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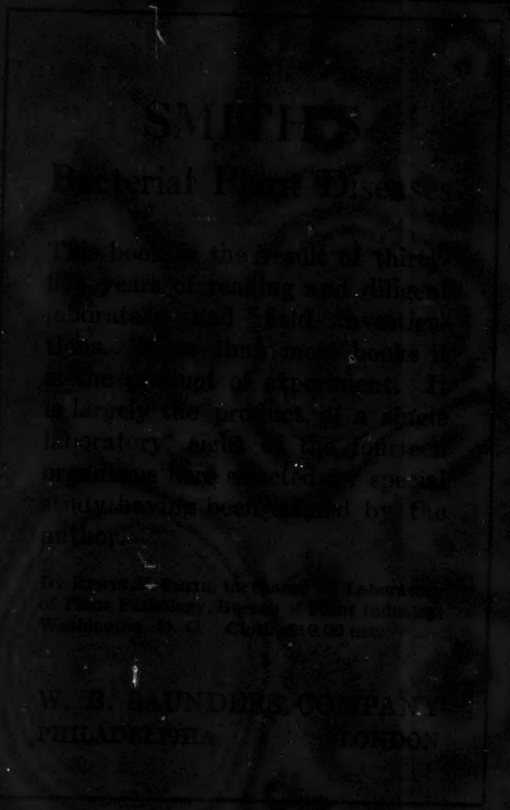


SMITH'S
Bacterial Plant Diseases

PHYTOPATHOLOGY

VOLUME XI NUMBER 7

JULY, 1921

PHOMA ON SWEET SORGHUM

ELIZABETH KOCH AND CAROLINE RUMBOLD

WITH PLATES IX TO XI AND THREE FIGURES IN THE TEXT

DISTRIBUTION AND OCCURRENCE OF THE DISEASE

On leaves. In 1917, this laboratory received some diseased sorghum plants, which had leaves infected with Phoma. The plants had been collected August 21 at Fort Smith, Arkansas; September 15 at Manhattan, Kansas; October 6, in Choctaw County, Mississippi, and on October 31, at Laredo, Texas. In 1918 the laboratory received a few sorghum plants collected September 6, at Ratcliffe, Arkansas, with leaves infected with Phoma. In 1920 three different lots of diseased seedlings were received from Arkansas. One lot was collected near Ursula on July 20, the other two lots from the neighborhood of Fort Smith, July 28. In one lot, which came from near Fort Smith, the seedlings had yellow leaves and well developed Phoma pycnidia were found in the discolored areas. The other two sets of plants had green leaves, but when cultures were made from them, were found infected with Phoma. On August 30, 1920, the junior writer collected Phoma infected leaves from the Black Amber, Early Amber, Planter's Friend, Silver Top, and Japanese Ribbon varieties of sweet sorghum growing on the Government farm at Arlington, Va.

On seeds. Mr. H. B. Cowgill, of the Office of Sugar-Plant Investigations, kindly furnished seed of varieties of sweet sorghum grown on the experimental plots at Fort Smith, Arkansas. During the winter of 1919-20, five varieties: Planter's Friend, Sapling, Collier, Orange and Red X were found infected with Phoma. Pycnidia were found also on the glumes of Planter's Friend and on the seed stems of Red X. During the winter of 1920-21 he furnished additional varieties of sweet sorghum seed from the same source. Examination showed Phoma fruiting on Albaugh Early, U. S. Colman, Fort Scott Colman, Fort Smith Colman, Farmer Jones, Honey, Indiana Amber, Minnesota Amber, Sugar Drip and McLean (1919 harvest). Pycnidia were found on the glumes of Red Amber, Minnesota Amber and Japanese Ribbon and the lemma of Silver Top seed.

In view of the widespread occurrence of the Phoma on sweet sorghum

and its persistence year after year, it was thought a more detailed knowledge of the life history of the fungus would be of value.

GENERAL APPEARANCE OF DISEASED SEED AND LEAF

To the unaided eye the fungus looked like a fine dust on the seed coat and on the leaf, giving them a dirty appearance. (A quick way of telling whether a leaf or seed was infected was to rub it off with the thumb. The dirt rubbed off, while the pycnidia, being embedded in the host tissues, stood out, plainly in view).

The seed did not show discolored spots, the pycnidia were scattered and often had formed on the epidermis in a line just above the edge of the glume. (Pl. XI.) Usually the infected areas on the leaves were yellow or tan colored and dry. These areas were irregular in shape, sometimes indefinite in outlines, sometimes bordered by black or red lines. (Plate IX.) Rarely mature infected leaves were normal in color. The infected leaves of inoculated seedlings usually turned yellow as the pycnidia formed. (Fig. 1.)

FIG. 1. LEAF OF AN EARLY AMBER SEEDLING INOCULATED WITH CONIDIA OF PHOMA INSIDIOSA FROM COLLIER. × 1¼.

INOCULATION EXPERIMENTS

Isolation of the fungus. The cultures were started from single conidia by means of poured plates. New cultures were always started from conidia,—not made by transfers of mycelium,—as it was found that the fungus ceased to produce fruits after repeated transfers of mycelium were made. The pycnidia produced in the cultures were transferred to sterile water, crushed and the resulting suspension of conidia used for inoculating plants.

Experimental material. Five varieties of the sorghum seed held in the laboratory in 1919-20, were found free of Phoma pycnidia: Early Rose, Silver Top, Early Amber, Folger and Japanese Ribbon. These varieties were used in the experimental work instead of those varieties which were found infected, as the writers hoped in this way to find an immune variety of sorghum. With one exception, the plants were inoculated while in the seedling stage. The seedlings used were either growing in pots in the greenhouse or in covered glass dishes in the laboratory.

Six strains of Phoma were used because at first it was thought that there might be a difference in the virulence of the strains or that certain of the varieties of sorghum might be immune from some of them. Five of the strains were obtained from pycnidia found growing on the seed coats of Planter's Friend, Red X, Collier, Orange, and Sapling. The sixth strain used, called So 303 1, came from pycnidia on the leaves of an Early Amber plant inoculated with a Phoma obtained from the leaves of a sorghum plant grown in Arkansas.

Method of inoculation. After the first experiments, all inoculations were made by spraying the plants by means of an atomizer with a water suspension of conidia.

Wounding the plants. In a preliminary experiment part of a set of seedlings were wounded by needle pricks just before spraying; the other part was not injured. All the plants, pricked and unpricked, became infected and pycnidia developed at the same time on both sets. This experiment was repeated, but instead of conidia, mycelium was used for inoculating purposes. Again all the plants pricked and unpricked became infected. The pycnidia were longer in forming on the plants inoculated with mycelium than those with conidia. After these experiments, all inoculations were made without previously wounding the plants.

Greenhouse experiments. Seed of the five varieties of sorghum used in the experiments were sown in pots. Thirteen pots were prepared for each strain of Phoma and one for a check. Each pot contained three plants of each variety growing in a row. While in the seedling stage the plants were sprayed with a water suspension of conidia. Check plants were sprayed with sterile water.

The first inoculation of the sorghum seedlings in the greenhouse appeared unsuccessful. Nine days after the spraying no signs of infection were evident. The weather then changed, becoming warmer and more humid. The plants, no longer in the seedling stage, were reinoculated. Eleven days later pycnidia had developed. All the checks were free from infection.

The experiment was repeated, exactly the same method being used. The seedlings were inoculated at the same time that the older sorghum plants of the first experiment were reinoculated. On the seedlings, pycnidia were visible four days after inoculation. No pycnidia developed on the checks.

Only those plants showing pycnidia on the leaves were counted as infected. The counts of the two experiments showed that all of the six strains of Phoma could infect each of the five varieties of sorghum, though not all the plants became diseased. After the count was taken, some of

the plants which showed no signs of infection were cut and placed in damp chambers in the laboratory. Gradually the leaves yellowed and Phoma pycnidia developed. In these two experiments the development of the pycnidia seemed to be influenced by the checking of the host plants' growth and in turn the checking of the vegetative growth of the invading fungus. The older set of sorghum plants required seven more days to develop pycnidia than the younger, and as the plants were growing in the same greenhouse under the same conditions, this delay must be laid to the slower reaction of the older plants to infection. The experiment was repeated, the same method being used. The final reading was taken thirty-five days after inoculation. In this experiment the sixty five sorghum seedlings of each variety used in the previous experiments, were depleted in number, due to accidents.

TABLE 1

Greenhouse Experiment: Plants growing in flower pots. Reading taken 35 days after inoculation of plants showing pycnidia on the leaves.

SORGHUM INOCULATED	INFECTION PRODUCED BY FOLLOWING STRAINS OF PHOMA[1]																SUMMARY OF ALL STRAINS	
	PLANTER'S FRIEND		RED X		COLLIER		ORANGE		SAPLING		SO 303 1		CHECK					
Early Amber	7/10	% 70	4/10	% 40	4/8	% 50	2/9	% 22	8/10	% 80	4/10	% 40	0/5	% 0	29/57	% 51
Silver Top	4/5	80	4/9	44	3/5	60	5/7	71	7/8	88	3/9	33	0/4	0	26/43	60
Folger	8/9	89	2/10	20	2/10	20	2/9	22	5/10	50	4/9	44	0/5	0	23/57	40
Japanese Ribbon	7/10	70	3/8	38	3/9	33	4/10	40	4/10	40	0/9	0	0/5	0	21/56	38
Early Rose	6/7	86	1/5	20	2/7	29	1/5	20	5/5	100	0/7	0	0/3	0	15/36	42

[1] Numerators of the fractions signify the number of infected plants. Denominators signify the number of plants inoculated.

These greenhouse experiments demonstrated the dependence of the fungus on warm and humid weather for vigorous growth. No conclusion could be drawn as to the degree of resistance or non-resistance to the fungus of the different varieties of sorghum. No variety was immune.

Laboratory Experiments. Sorghum seeds were germinated in blotters and the fresh, young seedlings transferred to sterile Petri dishes,—five in a dish. In each experiment thirteen dishes of each variety were prepared

—two for each strain of Phoma and one for a check. These were inoculated by spraying with an atomizer a water suspension of conidia. Check plants were sprayed with sterile water. The test was made four times, each time with similar results. The number of days required for pycnidia to develop varied according to weather conditions, but to a lesser degree than in the greenhouse. On these plants the pycnidia developed on roots and stems as well as on the leaves. The following table gives the combined results of three experiments performed under practically the same conditions of temperature and moisture and with readings taken at nearly the same intervals.

TABLE 2

Seedlings grown in covered glass dishes. Readings taken 29 days after inoculation Average of three laboratory experiments.

SORGHUM INOCULATED	NO. OF PLANTS FOR EACH STRAIN OF PHOMA	NUMBER OF PLANTS INFECTED BY STRAINS OF PHOMA FROM						CHECK[1]	TOTAL AMT. OF INFECTION BY ALL STRAINS	
		PLANTER'S FRIEND	RED X	COLLIER	ORANGE	SAPLING	SO 303 1			
										%
Early Amber	30	14	23	23	13	23	20	0/15	119/180	66
Silver Top	30	22	22	18	17	18	18	0/15	115/180	64
Folger	30	16	13	12	12	14	20	0/15	87/180	48
Japanese Ribbon	30	20	19	17	8	12	10	0/15	86/180	48
Early Rose	30	21	25	25	19	17	22	0/15	129/180	72

[1] Numerators of fractions signify the number of infected plants. Denominators signify the number of plants inoculated.

All of the varieties of sorghum became infected and all the strains of Phoma produced infection. Of the sorghums, Early Rose seemed the least resistant in these experiments, while Folger and Japanese Ribbon appeared most resistant. The seedlings showed this in a much more striking way than the numbers in the table indicate, Early Rose seedlings always being much more diseased than the Folger and Japanese Ribbon. The strains of Phoma showed differences in the amount of infection they produced in the different varieties of sorghum, but these differences were not uniform in each experiment. All the strains appeared alike as re-

gards their morphology and manner of growth on the different varieties of sorghum plants. It is believed that they are the same fungus found growing on different hosts, and the difference found in the amount of infection produced by them was probably due to their previous life history.

GERMINATION AND GROWTH OF DISEASED SEED

To determine the effect on germination of the presence of Phoma pycnidia on the sorghum seed, a comparative test was made of diseased and uninfected seed. There were four varieties of sorghum showing pycnidia on the seed coats, used in the test: Orange, Planter's Friend, Red X, and Sapling. Seed showing Phoma pycnidia and seed apparently clean of the fungus, were selected from the seed bags and without treatment of any kind, dropped into sterile Petri dishes on moist blotting paper. As shown in the following table the germination percentage of the infected seed is less than that of the clean. Some of the seed germinated and, later, pycnidia developed on the leaves of some of the resulting seedlings. Some of the seed did not germinate, but in the moist warm atmosphere of the glass dish developed mycelium and new pycnidia. The diseased seed of Planter's Friend produced clean seedlings. Of the checks, a Red X seedling showed pycnidia.

TABLE 3

Germination of diseased seed in Petri dishes

VARIETY OF SORGHUM SEED	INFECTED SEED				HEALTHY SEED			
	NO. OF SEEDS	NO. OF SEED GERMINATED	GERMINATED	INFECTION	NO. OF SEEDS	NO. OF SEED GERMINATED	GERMINATED	INFECTED
			%				%	
Orange	10	7	70	1 dead seed 2 seedlings	5	5	100	0
Planter's Friend	10	9	90	0	5	5	100	0
Red X	6	3	50	1 seedling 2 dead	5	5	100	1 seedling
Sapling	10	10	100	1 seedling	5	5	100	0

These results indicate the possibility of spreading the disease by means of infected seed and the loss in the field from the sowing of such seed. After the diseased seeds had germinated and passed safely through the early seedling stages, the fungus did not appear to affect severely the maturing of the sorghum plants. Diseased seeds (Planter's Friend) were germinated in pots in the greenhouse. Those that appeared above

ground grew into healthy looking plants and matured seeds which were found free from pycidnia.

OTHER PLANTS EXPOSED TO INFECTION OF THE FUNGUS.

The area in the United States in which sweet sorghum is cultivated has spread rapidly in the last few years. It is grown in the central and southern states and is an important crop in Alabama, Arkansas and Florida. In this region grain of forage sorghum, corn and sugar cane are crops of great fiscal importance. For this reason it was a point of some interest to see whether grain sorghum, sugar cane and corn were susceptible to the sweet sorghum Phoma.

Grain sorghum susceptible to sweet sorghum Phoma. A grain sorghum of the variety brown durra, named Yellow Jerusalem Corn was infected with two strains of sweet sorghum Phoma: the Phoma found on Red X seed and the Phoma originally found growing on sorghum leaves gathered in Arkansas (So 303 1). The Red X Phoma cultures had been growing for a year in the laboratory and So 303 1 for half a year. The grain sorghum seeds were germinated in blotters, the young seedlings transferred to sterile Petri dishes—five in a dish—and sprayed with water suspensions of conidia. There were 20 seedlings inoculated with each strain of Phoma and 10 check seedlings sprayed with sterile water. In 16 days the set of plants inoculated with Red X Phoma showed 7 seedlings which had developed pycnidia on leaves, stems and roots, while the set inoculated with So 303 1 showed visible infection on 4 seedlings in 18 days. The checks remained free from pycnidia.

Sugar cane and corn susceptible to the sorghum Phoma.—Some experiments were made in order to test the pathogenicity of the sorghum Phoma on the leaves of sugar cane and corn.

Four small sugar cane plants of the Louisiana Purple variety were used in the test. They were growing thriftily in pots in the tropical greenhouse. One plant was inoculated with the strain of Phoma taken from Planter's Friend seed, another with the Collier strain, and the third with So 303 1. The fourth plant, held as a check, was sprayed with sterile water. In two weeks pycnidia were found on the lower leaves of the plants sprayed with the Phoma from Planter's Friend and from Collier. The edges of the leaves had yellowed and pycnidia had formed in the dry leaf tissue. The plant inoculated with So 303 1 and the check remained free from the fungus. At the same time a search was made for signs of Phoma on the other sugar cane plants growing in the greenhouse, but no signs of the fungus were found. The infected cane plants were not checked in growth by the Phoma and there was no indication that the

fungus was more than slightly pathogenic to sugar cane. The Phoma fungus on the sugar cane leaves was recovered in the laboratory and was identical with the sorghum Phoma.

In the test made with corn (*Zea mays*) the variety used was U. S. Select 187. The same three strains of Phoma were used that were used in the sugar cane experiment. Two sets of corn seedlings growing in pots in the greenhouse and 2 sets of seedlings growing in glass dishes in the laboratory were used. Six plants were inoculated with each fungus culture and three plants were sprayed with water in each set. In the laboratory the leaves of the corn seedlings inoculated with the Collier strain of Phoma showed pycnidia in two weeks. In a month all three Phoma strains had produced pycnidia on two or more plants. The checks remained free of the fungus. In the greenhouse the seedlings showed no signs of Phoma pycnidia at the end of a month. There was no indication in this test that the sorghum Phoma was more than slightly injurious to the corn plants.

CROSS INOCULATION OF THE FUNGUS

Sorghum seedlings inoculated with Phoma from sugar cane. Folger, Early Rose, Japanese Ribbon, Early Amber, and Silver Top seedlings growing in glass dishes were inoculated with conidia from cultures made from pycnidia growing on sugar cane. This sugar cane had been inoculated with Phoma growing on sorghum seed (Planter's Friend). In two weeks Early Rose, Folger, and Early Amber seedlings showed pycnidia on the leaves, in a month all of the five varieties of sorghum showed infected plants. The checks remained free of Phoma.

Sorghum seedlings inoculated with Phoma from sorghum. A test parallel with the sugar cane Phoma was made with a culture of Phoma obtained from Early Rose sorghum which had been inoculated in the greenhouse with the conidia from pycnidia found on Sapling seed. The same five varieties of sorghum seedlings growing in glass dishes were sprayed with conidia. Two weeks afterward pycnidia were found on the leaves of some of the Early Rose, Folger, and Japanese Ribbon plants. In a month seedlings of every variety had become infected. The checks showed no pycnidia.

Sorghum seeds inoculated with Phoma from sorghum and sugar cane. Twenty sorghum seeds (Early Amber) of the 1920 harvest were sprayed with Phoma conidia derived from sorghum (Early Rose) which had been inoculated with Phoma taken from sorghum seed (Sapling).

At the same time another set of 20 seeds was sprayed with conidia taken from the sugar cane inoculated in the greenhouse. One half of the seed was scarred with a knife before inoculation, the other half not. The seeds were not sterilized but were kept in sterile Petri dishes. All the

seed germinated and all the scarred ones,—both check and inoculated, were covered with saprophytic fungi. The unscarred seed remained cleaner. In three weeks the seedlings and fungous mycelium had dried out and the mycelium was removed from the seeds with a needle. Pycnidia were found on some of the scarred and some of the unscratched seeds, both inoculated with the sorghum Phoma and with the sugar cane fungus. Examination of infected seed showed that the mycelium had penetrated the outer layer only of the seed coat.

DESCRIPTION OF THE FUNGUS ON ITS HOST.

Pycnidia. When walking through a field of mature sorghum, the Phoma infections were usually found on the edges and tips of the lower leaves of the stalks. The pycnidia were generally found in dry spots on the leaves, either scattered or in groups; often they developed in lines between the veins of the leaves. (Fig. 2.)

FIG 2. PYCNIDIA OF PHOMA INSIDIOSA ON A SOPGHUM LEAF.

They were at first covered by the epidermis, but later broke through. Occasionally the developing pycnidia were able to rip off and push up to some extent the cuticle of the tender sorghum seedlings which were growing in the laboratory, before it gave way to the steady pressure of the fruits. They varied greatly in size and shape; some globular, others lens shaped and occasionally compound pycnidia were produced with a single opening or ostiole. The ostiole varied in shape, sometimes it was a pore, the cells surrounding it a darker brown color than the pycnidial wall; on some pycnidia the ostiole was a well developed beak and there were shapes between these extremes. The mature pycnidial wall was dark brown and black when dried out. This variation in form was noticed especially in the pycnidia grown in the greenhouse and in laboratory cultures.

Mycelium. No perithecia were seen either on the host plants or in cultures. The mycelium in the tissue of the host plant was scant, even when pycnidia were developing. Sections of infected sorghum leaves showed that as a rule there was but one hypha in a leaf cell. The hypha was constricted when passing through the cell walls. The mycelium was found passing along the vascular bundles in the phloem cells. Cross sections of leaves usually showed the pycnidia developing next to a vein. Mycelium was never seen in the vessels. In the leaf tissue of sorghum seedlings inoculated and growing in Petri dishes, the fungus was found in all the cells except the vessels.

A search was made to see whether any of the hyphae growing from the conidia sprayed on the leaves entered the stomata. In only one case was this phenomenon seen, while in several cases they were observed crossing the stomatal opening. Hyphae have been found entering a break in the leaf epidermis. Whether this break was due to fungus action or to a mechanical accident is not definitely known. In view of the repeated successful inoculations by spraying conidia on uninjured seedlings and of a phenomenon observed in the germinating conidia, it is thought that the hyphae of the germinating conidia possess the power to penetrate the epidermis of the sorghum plant when it is in a tender or weakened condition, and that the fungus invades the host plant in this manner as well as by entering through accidental wounds in the epidermis.

CULTURAL CHARACTERISTICS OF THE FUNGUS

Mycelium. The variability of Phoma in culture makes it difficult to give a satisfactory description of its appearance. Its characteristics, either morphological or physiological, are not well fixed. The mycelium in cultures on agar was usually rather sparse, but by repeated transfers of mycelium a relatively abundant sterile growth could be cultivated. At first the hyphae were white and sparsely septate. After a short time the submerged hyphae became brown and short celled, in certain circumstances and on certain media they became olive-green or pink colored. The aerial mycelium was usually white or grey colored with occasional pink colored clumps. The dark color of the cultures was sometimes caused by colored hyphae, sometimes by the presence of innumerable black pycnidia. When growing on a favorable medium without interference, few or no pycnidia developed. If the hyphal growth was stopped either by cutting out a part of the culture, or by the meeting of growing hyphae of neighboring colonies, a row of pycnidia developed along the cut edge or the border between the colonies. Advantage could be taken of this tendency of the fungus and when fresh pycnidia were wanted quickly, a poured plate was made with thickly sown conidia. The germ-

inating conidia rapidly produced abundant pycnidia and scant mycelium.

Chlamydospores. The chlamydospores were variable in size and shape. Their occurrence appeared to bear some relation to available nutriment, as they were usually present when few or no pycnidia were forming. They were both terminal and intercalary. Often the pink colored clumps of aerial mycelium were composed of hyphae with innumerable chlamydospores.

Effect of light and medium on the mycelium. A characteristic, soon noticed, of the fungus when growing on cornmeal agar was a pink discoloration of the medium, which the mycelium produced when growing in the light. This was especially marked in poured plates exposed to the light just after germination of the conidia. Each conidium was the center of a pink spot. A steadily-growing Petri dish culture, undisturbed for several days, showed a series of concentric pink and white circles radiating from the conidium. A count showed that the white circles marked the spaces traversed by the hyphae during the night, the pink circles during the day. This pink color faded as the culture aged. Old and slowly growing mycelium did not produce the color. All the strains used in these experiments showed at first this pink discoloration. During the summer months with a mean temperature of 80° F., the life cycle of the fungus was completed in about two days, and the pink color was always found on cornmeal agar when growing in the light. In the autumn scattered colonies were noticed in the plates which produced no discoloration of the medium, but developed pycnidia more quickly and abundantly than the pink colonies. At first it was thought these might be contaminations, then that they were caused by the cool, dry atmosphere in the room which had caused a change in the life processes of the fungus, for now it frequently took a conidium two days to germinate and two weeks to complete its life cycle. A test was made with a single mature pycnidium taken from a Sapling culture two months old. The fruit was crushed and the conidia plated in cornmeal agar in a series of Petri dishes. Half the plates were covered from the light, half exposed. One set of the cultures grew in temperature of 68° to 70° F., the other set in a temperature of 79° to 81° F. Both kinds of colonies developed in all the plates. Experiments showed that all the strains of Phoma developed both kinds of colonies, though in different degree. At one extreme the Red X fungus produced during the winter months innumerable pycnidia on a scant mycelium and no stain on cornmeal agar even when growing in the light (Plate X, fig. 1), while the So 303 1 strain (Plate X, fig. 2), continued to develop the pink staining mycelium even in the dark.—Though cultures were developed from conidia thickly sown and grown in the dark which produced the nonstaining, pycnidia-developing mycelium, even

then occasional pink colonies developed in these plates. Plate X illustrates this difference in the appearance of the cultures. These cultures were plated in cornmeal agar at the same time and kept under the same conditions.

TABLE 4

The production of color, pycnidia and chlamydospores in cornmeal agar poured plate cultures by Phoma insidiosa obtained from various sources.

SOURCE OF CULTURE	PART FROM WHICH CULTURE WAS MADE	FIRST PLATED	COLOR PRODUCED IN AGAR				PYCNIDIA	CHLAMYDOSPORES
			AT FIRST PLATING	OCT. 20' CULTURES, 1½ MOS. OLD	Feb. 1921			
					3 MO. OLD	1 YR. OLD		
Planter's Friend	Seed coat (1919 seed)	Feb. '20	Pink	No color		No color[1] Pink	Numerous	Present
Red X	"	Feb. '20	"	"	No color	No color[1] Pink	"	"
So 303 I	Early Amber leaves inoc. with So 303	Aug. '20	"	Pink	Pink	....	Less numer-	"
Sapling	Seed Coat (1919 seed)	Aug. '20	"	No color	No color[2] pink	....	Numerous	"
Orange	"	Aug. '20	"	"	....	....	"	Not examined
Collier	"	Aug. '20	"	"	....	....	"	Not examined
McLean	"	Feb. '21	"	....	....	....	"	Present

[1] Two kinds of colonies, one producing pink and one no color. (See plate X.)

[2] A fresh culture from a 1919 seed coat planted in Feb., 1921, fruited sparsely after three weeks. The conidia from this fresh culture developed two kinds of colonies, one producing a pink stain on agar and one no color.

The fungus seemed to show a tendency to a summer and a winter phase of growth. In the summer phase the fungus developed a mycelium which was sensitive to the light, while in winter it developed innumerable pycnidia on a scant mycelium insensitive to light.

The brilliancy of the stain which developed, varied with the medium. The deepest color appeared in dextrose beef agar, where the hyphae also were colored a deep red. Next in vividness was the beef juice agar, oatmeal agar, dextrose litmus agar and oxalic acid beef juice agar. So 303

1 cultures on potato cylinders developed a white mycelium from which oozed drops of red liquid. The red coloring matter in the hyphae and the agar was partly soluble in alcohol.

Conidia. The conidia were hyaline, one-celled. Young conidia, when freshly ejected from the pycnidium, appeared to be filled with solid protoplasm, later two, rarely three, globules formed. Mature conidia usually showed two globules when ejected. In shape they were sub-cylindrical, with blunt sometimes tapering ends. (Fig. 3.)

FIG. 3 CONIDIA OF PHOMA INSIDIOSA GERMINATED IN WAER. X 625.

The germination of the conidia was observed in Van Tieghem cells. The conidias welled to about three times their original size before the germinating hyphae protruded. (Fig. 3 b.) One or two hyphae appeared, usually at the ends of the conidium, but sometimes at the sides. (Fig. 3, c) Frequently a cross well developed in the conidium before the germinating hypha was produced. (Fig. 3, d.) The conidia germinated readily in distilled water, but growth ceased soon after germination. The growing tip of the germinating hypha was always dense with protoplasm and highly refractive when observed through the microscope. In the water cultures a phenomenon was observed which was thought of significance with regard to the penetration of the epidermal cells of the sorghum leaf by the fungus. The tips of the germinating hyphae of those conidia lying next to the cover glass fastened themselves to it for a short period of time. The tips rounded themselves against the glass as though using suction and in the center a dense, shining, round spot of protoplasm was observed. (Fig. 3, c.)

Pycnidia and condia varied in size. The following table shows the mean measurements of fruits and spores taken from different sub-strata.

TAXONOMY

For the present the writers regard the Phoma gathered from the different varieties of sweet sorghum seeds and leaves as the same fungus. The cultures arising from these different sources have been kept carefully separated, in order that variations in the different strains as regards morphology and manner of growth on the different sorghums,

TABLE 5

Measurements in microns of pycnidia and conidia on seeds, leaves and in cultures

SOURCE	PYCOIDA			CONIDIA		
	NO MEASURED	RANGE IN μ	MEAN SIZE IN μ	NO MEASURED	RANGE IN μ	MEAN SIZE IN
Planters' Friend—Seed coat	10	98 to 252	159	10	2.6 to 3.5 × 5.3 to 7.0	3.3 × 6.6
G.ume	10	45 " 108	70			
Leaf—(Greenhouse inocu.ation to Early Amber.)	5	45 " 99	76			
In culture (cornmeal agar)	10	81 to 185	143	10	2.6 to 3.5 × 4.4 to 7.0	3.3 × 5.0
So 303—Leaf—Field infection	20	56 " 168	107	20	2.7 " 4.4 × 6.5 " 8.8	3.5 × 6.3
So 303 1—Laboratory inoculation to Ear.y Amber	4	90 " 162	124	10	1.4 " 3.2 × 3.8 " 7.8	2.7 × 6.0
Leaf—Laboratory inoculation to Brown Durra	10	36 " 90	53	10	3.5 " 4.4 × 4.4 " 7.0	3.6 × 5.6
In culture (cornmeal agar)	10	45 " 90	67	10	1.8 " 3.5 × 4.4 " 7.0	3.0 × 5.3
So 303 1 to Brown Durra—In culture (cornmeal agar)	10	41 " 126	59	10	2.6 " 3.5 × 4.4 " 7.0	3.2 × 5.5
Red X—Leaf—Laboratory inoculation to Brown Durra	10	45 " 90	66	10	3.5 " 3.9 × 5.2 " 7.0	3.5 × 6.3
In culture (cornmeal agar)	10	54 " 99	80	10	2.6 " 3.9 × 5.0 " 7.9	3.5 × 6.7
Red X to Brown Durra—In culture (cornmeal agar)	10	45 " 90	64	10	" 3.5 × 5.0 " 7.9	3.5 × 6.5
Sapling—Leaf—Greenhouse inoculation to Early Rose	6	41 " 117	73			
In culture (cornmeal agar)	32	41 " 224	169	10	2.6 " 3.9 × 4.4 " 6.1	3.5 × 5.3
Orange—In culture (cornmeal agar)	11	27 " 144	79			
Collier—Leaf—Laboratory inoculation to Early Rose	10	27 " 16	89			
Silver Top—Lemma	10	72 " 171	142			
Albaugh Early—Seed coat	10	84 " 168	132			
McLean—In culture (cornmeal agar)	10	81 " 162	122	10	2.6 to 4.4 × 4.4 to 7.0	3.5 × 5.6
Mean	248	27 to 16	012	130	1.4 to 4.4 × 3.5 to 8.8	3.4 × 5.9

cane, corn, grass and on artificial media might be noticed. Such variations as have been found have been described. They are not thought to be sufficient to warrant special naming of the strains.

The first published report of Phoma on sorghum seed, which could be found was that by F. Tassi in the Bulletino del LaboratorioBotanico della R. Universiti di Siena, Vol. 1, p. 8, 1897.

Phoma insidiosa F. Tass.

"Maculis cinereo-albicantibus epidermidem seminum late ambientibus: peritheciis sparsis, vel sub-gregariis, piceis, immersis, primo epidermide tenuissima sub-velatis, ostiolo late pertusis, 70–80 μ diam.; sporulis innumeris, ellipticis initio continuis, tarde minute 2-guttulatis, vel granulosis, hyalinis, 6 × 2–2½ μ. (Tab. IX, fig. 8.)

Hab. in seminibus *Sorghi vulgaris* B. *Dourah* ex Abyssinia (1897)."

Phoma insidiosa F. Tass.

Spots ash colored to somewhat white spreading over the epidermis of seeds; pycnidia sparse or somewhat grouped, black, immersed, at first somewhat covered by the epidermis, ostiole somewhat protrudent, 70–80 μ diameter, innumerable spores, elliptical, at first continuous, later two minute globules, or granular, hyaline, 6 × 2–2½ μ.

Collected on seed of *Sorghum vulgaris* B. durra, in Abyssinia 1897.

Tassi found *Phoma insidiosa* growing on durra sorghum seed. The American fungus was first seen on sweet sorghum seed and leaves, and only later found on grain sorghum leaves. But as brown durra sorghum seedlings were infected by the sweet sorghum Phoma, as well as sugar cane and maize seedlings, it was thought the description of *Phoma insidiosa* corresponded sufficiently to apply to the American fungus. The mean measurement of the diameter of the American pycnidia is larger, but the Tassi measurements fall within the extreme measurements. The conidia of the American fungus also are larger, but as some of them measured 6 × 2.7 μ, these measurements were not deemed sufficiently greater to warrant giving the fungus another name. The name *Phoma insidiosa* Tassi is, therefore, tentatively given the fungus described in this paper, until a type specimen of the African fungus has been examined.

VITALTY OF PHOMA IN NATURE AND IN CULTURES

The Phoma found on sweet sorghum leaves collected in 1917 and 1918 and kept in the laboratory was dead when tested in November, 1920. Examination showed that most of the pycnidia ontained no spores. The few found did not germinate when tested.

Conidia taken from pynidia found on sorghum seed readily germinat-

ed and developed fruits for a year after harvest. The cultures used in many of the experiments were started in August 1920 from diseased seed harvested in 1919. By January 1921, however, the fungus on the seed showed a decided decrease in vitality. Very few conidia would germinate and those which grew produced mycelium and few pycnidia.

Conidia were still viable after thirteen months of desiccation in Petri dish cultures.

Little definite information can be given as to the economic importance of the sorghum Phoma. It evidently is widespread in occurrence in sorghum growing regions and appears persistently year after year. It is a facultative parasitic fungus as shown by its presence on all parts of the shoot of the sweet sorghum plant from the seedling stage to maturity and its ability to infect sugar cane, corn and grain sorghum. After watching the fungus's great adaptability as demonstrated in culture, the senior writer believes a search among the wild grasses growing in the sorghum territory will show the fungus growing on a number of them.

Mr. H. B. Cowgill, reports there was "difficulty in obtaining a stand of the sweet sorghums, especially in the early spring, on the experimental plots at South Fort Smith, Arkansas. Each season since the inception of this work there in 1918, certain varieties appear more or less susceptible to an apparent 'damping-off' following cool, wet weather. The five varieties of sweet sorghum grown extensively in the vicinity show relative susceptibility to the damping-off of the seedlings in the following order: Red X, Coleman, Indiana Amber, Folger's Early and Japanese Ribbon."

SUMMARY

Phoma infected sorghum has been collected in the different sorghum-growing states during the last five years.

The fungus has been found on the leaves of seedlings and the leaves, seed heads and seed of mature sweet sorghum plants.

It remains viable on seed for a year. The vitality is poor at the end of two years.

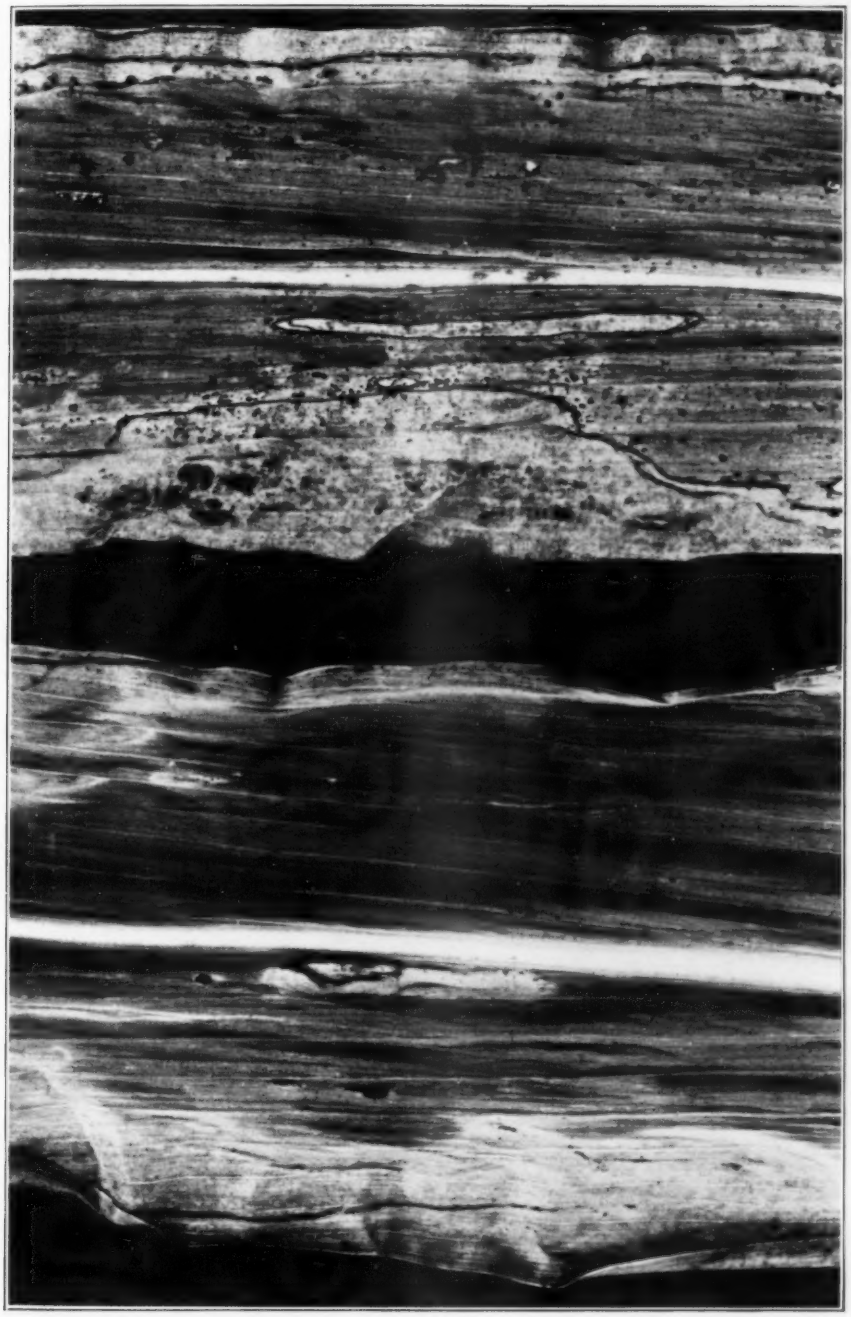
It has not been found viable on dry infected leaves two years old.

Of the eleven varieties of sorghum examined and experimented with, no variety has been found which is immune from the fungus.

The fungus can grow on the leaves and stems of the seedlings of the forage sorghum, Brown Durra.

It can also grow on sugar cane and corn seedlings, but is of slight pathogenicity.

SUGAR PLANT INVESTIGATIONS
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.



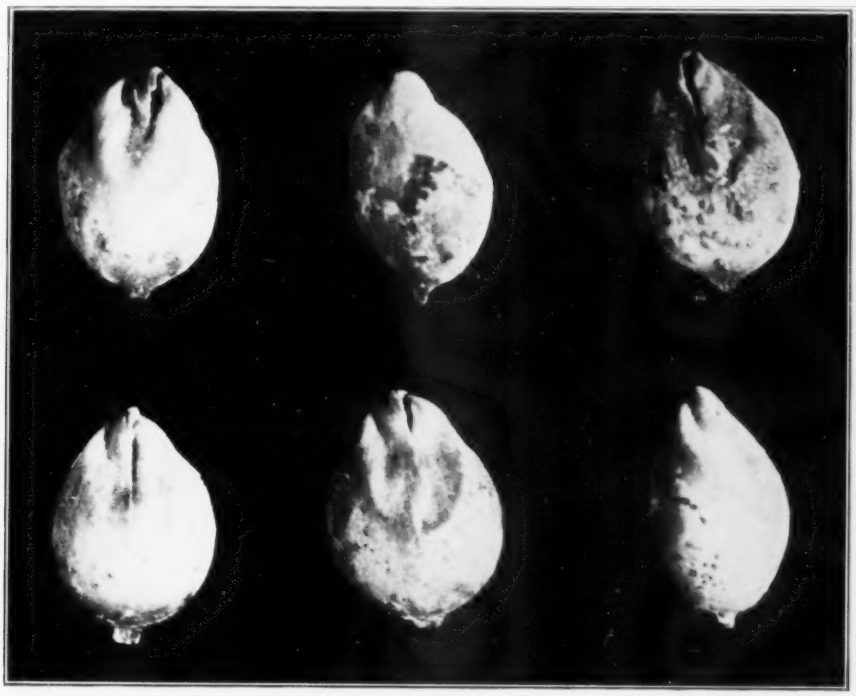
PHOMA LESIONS ON SORGHUM LEAVES

Upper leaf: lesion with indefinite outline on variety Silver Top.
Lower leaf: lesion with definite dark outline on variety Early Amber.

PETRI DISH CULTURES OF PHOMA INSIDIOSA

Fig. A. Culture from sorghum variety Red X producing innumerable pycnidia and no stain in corn meal agar.

Fig. B. Culture from sorghum So 303 I producing pink stain and few pycnidia in corn meal agar.



PYCNIDIA OF PHOMA INSIDIOSA ON SEEDS OF SORGHUM VARIETY PLANTER'S FRIEND.

THE RELATION OF SPORE LOAD TO THE PER CENT OF STINKING SMUT APPEARING IN THE CROP.

F. D. HEALD

INTRODUCTION

The value of the quantitative estimation of the number of smut spores carried by seed wheat was first pointed out by Bolley[1] in 1897, and this was given further consideration by Cobb[2] in 1904 and 1905. Although the centrifuge and counting cell have been used for determining the presence or absence of spores of disease producing fungi on seed, the direct relation of the spore load carried by each individual grain of wheat to the amount of smut appearing in the crop seems to have received but little attention. Cobb in 1904 wrote as follows: "The amount of smut that can be tolerated can only be determined by sowing through a series of seasons quantitatively examined seed presenting the necessary degrees of infection, and subsequently examining the resulting crops . . . I would call the attention of experiments to the absence at the present time of any such data."

The determination of the spore load can be of direct value to the farmer, first, as a guide in the selection of wheat for seed purposes, and second so that he may know whether seed treatment is necessary or will be a paying procedure.

There is never any doubt that visibly smutted seed is carrying large numbers of smut spores attached to each individual grain, but it is also known that apparently clean seed may be carrying large numbers of spores. A quantitative microscopic analysis can determine which of several apparently smut-free samples contains the smallest number of spores, and this knowledge could be used by a farmer to good advantage in the selection of his seed. On the basis of present information the sample showing the least smut would naturally receive first choice, but we should be forced to recommend seed treatment for all except smut-free seed. If however, we have available data showing the relation of the spore load to the amount of smut appearing in the crop, it should be possble to predict with reasonable accuracy the approximate per cent of

[1]Bolley, H. L. The use of the centrifuge in diagnosing plant diseases. Proc. Soc. for the Prom. of Agr. Science **23**: 82–85. 1902.

[2]Cobb, N. A. Quantitative estimation of disease spores. Agr. Gaz. New South Wales **15**: 670—680. 1904.

———— Quantitative estimation of bunt in seed-wheat. Agr. Gaz. New South Wales **16**: 1113–1117. 1905.

smut that would appear in any crop provided seed disinfection is not practiced.

Considering the enormous acreage of wheat we can be reasonably certain that much seed wheat is disinfected needlessly. This not only involves the cost of seed disinfection which reaches a high figure each year, but seed treatments cause very pronounced reductions in germination, giving poor stands or requiring the use of larger amounts of seed. Seed treatment, in the absence of definite information, has been regarded as "an insurance" or a "playing safe," but with microscopic analyses and field data to back them, it can be put upon a more scientific basis.

The present paper will present some of the results which have been obtained during the last few years in our study of the bunt (Tilletia tritici) problem in the Pacific Northwest, so far as they are related to the spore-load.

THE NUMBER OF SPORES PER GRAIN

The maximum per cent of smut found in any fields under farm operation during the past six years has not exceeded 88. It is needless to say say that with 88 per cent of all the heads smutted, the grain from such a field would be carrying about the maximum load of smut. In this extreme case each individual grain might be carrying 50,000 to 150,000 spores, but the actual number will depend in part upon the extent to which the smut balls break during the threshing operation. In this connection it may be noted that a single smut ball contains millions of spores. In one analysis Cobb estimated the spore content of a smut ball as 8,000,000. Numerous analyses made of small and large smut balls collected in the vicinity of Pullman have shown 6,000,000 to 9,000,000 spores. The spore-load shown by field samples may vary from the maximum given above to only a few spores per grain, or in certain sections the wheat has been found to be practically smut-free.

RELATION OF THE WEIGHT OF SMUT USED TO THE PER CENT OF SMUT APPEARING IN THE CROP

In our first studies of spore-load, the amount of smut used was determined by weight using 0.01 to 3 or 4 grams per 100 grams of seed. The smutting was accomplished by adding the given weights of pulverized smut or smut dust to the wheat samples and shaking the seed and smut in a bottle so as to secure as nearly as possible a uniform distribution of the spores over the surface of the grain.

The smut used in our tests has been stored in a cool dry room or in the grain room, in bundles collected at harvest time. Just before using, the smutted heads were ground with an Enterprise food chopper and the chaff

sifted out. In all infection tests smut from the previous harvest has been used, so as to secure maximum germination.

In these first tests no analyses were made to determine the number of spores carried by each grain of wheat. From table 1 it may be noted that 0.5–3 grams was required to produce maximum smutting. From these results it seems evident that Kirchner,[1] who used one gram of smut

TABLE 1

howing relation of weight of smut per 100 grams of seed to per cent. of smut in the crop

WEIGHT OF SMUT PER 100 GRAMS OF WHEAT	PER CENT. OF PLANTS SMUTTED	PER CENT. OF HEADS SMUTTED
EARLY WILBUR, SEEDED APRIL 15, 1915		
None	0.2	0.2
0.01 grams	0.6	0.3
0.1 "	4.3	1.6
0.25 "	5.4	2.4
0.5 "	17.5	8.5
1.0 "	28.1	15.4
2.0 "	17.5	8.5
3.0 "	36.9	23.5
EARLY WILBUR, SEEDED APRIL 22, 1916		
0.5 grams		65.7
1.25 "		73.0
4.00 "		81.9
EARLY WILBUR, SEEDED MAY 1, 1916.		
0.5 gram,		9.95
1.25 "		65.82
4.00 "		77.98
HYBRID 143, SEEDED OCT., 1916		
0 grams		9.2
0.01 "		18.0
0.1 "		31.1
0.5 "		55.5
1.0 "		55.9
2.0 "		34.2

[1]Kirchner, O. Uber die Empfänglichkeit vershiedener Weizensorten für Steinrankheit. Frühlings' Landw. Zeitung **55**: 781—794.

to 15 grams of seed in testing the comparative resistance of wheat varieties to smut, used much more smut than was necessary to secure maximum infection.

THE RELATION OF SPORE LOAD OF NATURALLY SMUTTED SEED TO THE PERCENT OF SMUT IN THE CROP.

In the fall of 1917 a number of farm samples of Bluestem and Marquis wheat were obtained and kept through the winter in cool storage as near comparable to farm conditions as possible. Analyses were made to determine the average number of smut spores per grain of wheat and duplicate plantings were made on both north and south slopes.

The method of making the spore counts was the one that has been employed in all of the later analyses. Since it differs somewhat from the centrifuge methods described by Bolley and by Cobb, a brief description will be presented.

TABLE 2

Showing relation of spore load of untreated naturally smutted farm seed to the per cent. of smut in the crop

PLANTED 3–30–18

SAMPLE NUMBER	VARIETY	NO. OF SPORES PER GRAIN OF WHEAT	PER CENT. OF SMUTTED PLANTS	
			SOUTH SLOPE	NORTH SLOPE
A 1	Bluestem	1015	1.29	1.29
A 2	"	562	3.63	7.42
A 3	"	47	3.17	8.99
A 4	"	15	0	0.43
A 5	"	141	2.45	8.61
A 6	"	31	0.51	0.60
B 1	Marquis	23	0	0.45
B 2	"	200	0.50	1.94
B 3	"	468	0.63	0
B 4	"	62	0	0
B 5	"	78	0	0
B 6	"	23	0	0
B 7	"	117	0.92	0
B 8	"	190	0	0.62
B 9	"	8	0	0
B 10	"	8	0.61	0
B 11	"	8	0	0
B 12	"	32	0	0

1. Count out 300 whole grains of wheat.
2. Put into 100 cc. of spore-free water in an Erlenmeyer flask.
3. Shake frequently for one-half hour.
4. Using the Levy counting cell and mechanical stage, make 5 spore counts, using the average number of spores as the spore content of the unit volume of the cell $\frac{2}{3}$cu. millimeter).
5. Compute the average number of spores carried by each grain of wheat.

This method was compared with the centrifuge method and was found to be equally accurate, hence it was adopted for general use since it was less troublesome.

The results obtained for the 1918 plantings are shown in table 2. Simmilar analyses and planting tests were made in the Spring of 1920 using a large number of varieties as shown in table 3.

TABLE 3

Showing the relation of the spore load of naturally smutted, untreated farm seed to the per cent. of smut in the crop

PLANTED SPRING, 1920

	NO. OF SEED LOTS	NO. OF SPORES PER GRAIN	PER CENT. SMUTTED PLANTS	
			NORTH SLOPE	SOUTH SLOPE
Marquis	1	0	0	0
"	4	18–83	0	0
"	8	208–374	0	0
"	3	478–842	1.0 (478)	0
Early Baart	6	0	0	0
" "	2	84-156	0	0
" "	1	47	1.87	0
" "	1	508	0	0
" "	1	2339	1.96	1.01
Hybrid 143	1	87	0	0
" 143	1	645	0	0
" 143	1	1185	2.00	1.35
" 143	1	4180	5.49	2.00
" 143	1	43.78	2.52	2.00
" 143	1	8188	0	2.00
Jenkins Club	1	779	1.05	0
"	1	1070	2.22	0
"	1	6087	0	0
"	1	9505	2.52	1.74
"	1	12167	3.00	2.00
Blue stem	4	176–1320	0–1.43	0–1.00
Red chaff	4	364–852	0–1.43	0–0.88

Since the amount of smut shown in spring wheat under ordinary farm conditions has been rather low, especially during the last few years, it is not surprising that seed samples taken at random would give rather low spore counts. The samples varied from those smut-free to those showing a maximum of 12167 spores per grain. In these samples, those which were smut-free, produced no smut in the field plantings, while some showing an evident spore-load, also remained smut-free in the planting test. In general however we can note a relation between the number of smut spores carried by the seed and the per cent of smut appearing in the crop. No uniformity could be expected by comparing a series of samples collected at widely different points on account of the diverse conditions to which the grain may have been subjected prior to being assembled for our tests. With but few exceptions the plantings on the north slopes always gave a higher per cent of smut than plantings on the south slope.

By making use of the seed from a series of experimental plots used for another purpose it was possible to obtain very heavily smutted seed of Hybrid 143 and Jones' Winter Fife. The fields of Hybrid 143 which furnished the naturally smutted seed showed 4.83 to 94.91 per cent of the heads smutted, while the Winter Fife plots showed 0.49 to 82.36 per cent of all the heads smutted. The 10 lots of wheat threshed from each variety therefore showed a wide range of spore-loads.

In order to overcome the possible error from smut infections from spores in the soil, the plats selected for the planting test was covered with a layer of straw 4 to 6 inches thick and then fired. The ground was then raked and the seed planted at once. Previous experience had shown that this treatment was sufficient to kill the wind-blown smut that might have been present in the surface layers of the soil. A duplicate planting of the Hybrid 143 was made in the spring. The results are shown in tables 4 and 5. The relation between spore-load and per cent of smut in the crop does not seem to be as regular and proportional as might be expected, but with some fluctuations there is a gradual increase in the per cent of smut with increase in the number of spores carried by each grain. Even the seed lots showing the highest spore-load did not produce maximum smutting. It seems rather difficult to understand why a spore-load of 40,000 spores per grain should but little more than double the amount of smut produced by a spore-load of 4500. Why will a spore-load of a thousand or more spores per grain not produce maximum smutting? Theoretically it would seem that such a behavior shold be true.

THE RELATION OF SPORE LOAD OF ARTIFICIALLY SMUTTED WHEAT TO THE PER CENT OF SMUT IN THE CROP

Since it was not possible to work with a large number of varieties, a

TABLE 4

Showing the relation of spore load of naturally smutted untreated Jones Winter Fife to the per cent. of smut in the crop

LOT NO.	PER CENT. OF SMUT HEADS IN CROP FURNISHING THE SEED	NUMBER OF SPORES PER GRAIN	PER CENT. SMUTTED PLANTS
1	8.10	833	23.66
2	5.16	937	17.01
3	12.35	3437	23.21
4	17.66	4583	31.61
5	0.49	937	10.40
6	2.20	833	12.95
7	2.43	416	8.87
8	0.69	625	12.21
9	82.05	40104	70.5
10	82.36	45416	73.04
11	Control, no smut	0	0

very resistant variety Marquise, and a very susceptible variety, Jenkins' Club, were selected for test in our spring plantings. The smutting was accomplished in the same manner as noted for the earlier studies on spore-load according to weight, except in the case of the smaller weights of smut. For these, in order to insure an equal distribution of the spores over the seed, the smut was suspended in water and sprayed onto the seed with an atomizer. The results are shown in table 6.

With but little fluctuation the results show a gradual increase in the per cent of smut in the crop with the increase in the spore-load, up to an amount (0.5 gram or about 35000 spores) necessary to produce maximum smutting. The comparison of the results with Marquis and Jenkins' Club brings out a number of interesting features. It is worthy of note that Marquis remained smutfree with 104 to 542 spores per grain, while Jenkins Club produced up to nearly 10 per cent of smutted plants with nearly similar spore-loads. In Jenkins' Club, for example, a spore-load of 5333 spores produced 60.79 per cent of smut while increasing the spore-load to 20687 spores per grain only increased the per cent of smutted plants to 66.23.

A comparison of the results from artificially smutted seed with the results of farm smutted seed as shown in tables 2 and 3, indicate that similar spore-loads do not give similar per cents of smut, but the per cent of smut appearing in the crop is much smaller in the farm infected seed than would be obtained from similar spore-loads of artificially infected seed. This is probably explained by the lessened viability of the spores

which have passed through the winter period on the surface of the seed, over those which have passed the winter in unbroken smut balls. In the artificial smutting the seed was smutted a short time before planting, using newly ground or pulverized smut.

TABLE 5

Showing the relation of the spore load of naturally smutted, untreated Hybrid 143 wheat to the per cent. of smut in the crop

LOT NUMBER	PER CENT. OF SMUT HEADS IN CROP FURNISHING THE SEED	NUMBER OF SPORES PER GRAIN	PER CENT. OF SMUTTED PLANTS	
			FALL SEEDED	SPRING SEEDED
1	78.10	24062	71.11	69.93
2	32.87	4271	47.10	26.89
3	54.17	4166	64.79	57.75
4	4.83	2396	33.82	21.05
5	61.10	17187	69.86	40.57
6	36.41	4687	41.76	23.88
7	38.13	8208	66.18	45.29
8	16.39	3229	32.20	27.53
9	94.91	38333	65.82	72.18
10	93.24	?	69.63	73.25
11	0	0	0	0

It seems to have been the general opinion of plant pathologists that infection of a wheat plant with bunt might be accomplished from a single spore, but our results seen opposed to such an idea. Food for thought shold be found in the fact that a considerable number of spores per grain, may not be sufficient to cause any infection. At present two possible explanations may be suggested. Either what we may term a multiple infection occurs, that is, an infection in which a number of spores participate, or there is a chemical mass effect due to numbers of spores, and infection may then be from a single infection thread.

SUMMARY

1. Single smut balls of *Tilletia tritici* contain from 6–9 million spores.

2. The number of smut spores carried by a single grain of normal wheat is designated as its spore-load.

3. The spore-load of wheat due to smutting during the threshing operation has varied from 0 to a maximum of 45416 in the various tests. Smut-free wheat is rare except from the drier sections of the state.

4. In artificial smutting at least 0.5 gram of powdered smut well distributed to each 100 grams of seed is necessary to produce maximum smutting. An increase in weight of smut used up to three grams per

hundred of seed has in some cases slightly increased the per cent of smut appearing in the crop.

5. The use of the Levy counting cell alone has given as satisfactory determinations of the spore-load as the centrifuge and counting cell combined.

TABLE 6

Showing the relation of the spore load of artificially smutted, untr ated spring wheat to the per cent. of smut in the crop

MARQUIS

WEIGHT OF SMUT PER 100 GRAMS WHEAT	NUMBER OF SPORES PER GRAIN	PER CENT. OF SMUTTED PLANTS	PER CENT OF SMUTTED HEADS
0.00 grams	104	0	0
0.005 "	333	0	0
0.01 "	542	0	0
0.1 "	5043	7.31	1.79
0.25 "	19687	15.21	2.88
0.5 "	34937	1.02	0.29
1.0 "	59229	24.81	8.22
2.0 "	96958	56.32	16.43
3.0 "	183375	15.49	8.74

JENKINS' CLUB

0.0 grams	104	0.49	1.80
0.005 "	458	3.63	2.45
0.01 "	533	9.52	5.99
0.1 "	5333	60.79	32.80
0.25 "	20687	66.23	43.77
0.5 "	36770	90.82	84.65
1.0 "	65229	94.43	92.92
2.0 "	161971	93.38	92.55
3.0 "	164208	98.88	94.58

6. In artificial smutting a spore-load of 36,000-150,000 has been required to produce the maximum per cent of smut in the crop.

7. The per cent of smut appearing in the crop increases with the spore-load but less rapidly.

8. The per cent of smut appearing in naturally smutted spring wheat is less than would be indicated by the spore-load as based on plantings of artificially smutted samples.

9. Planting tests of samples of spring wheat with known spore-load have made it possible to estimate the approximate amount of smut that will appear in a crop if the seed is planted without disinfection. This does not apply towinter wheat in the Palouse country since soil contamination is general and variable there.

10. With small spore-loads, certain varieties of spring wheat have remained smut-free, while others have given such a low per cent of smut as to make seed disinfection of doubtful value. Our tests of Marquis indicate that this variety will rarely require seed treatment.

11. The spore-load that will produce an appreciable amount of smut (9.52) in a susceptible variety (Jenkins' Club) has given a smut-free crop in a resistant variety (Marquis).

12. The relation of spore-load to the per cent of smut appearing in the crop indicate either that a multiple infection occurs or that there is a chemical mass effect due to numbers of spores.

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THE DECAY OF SWEET POTATOES (IPOMOEA BATATAS) PRODUCED BY DIFFERENT SPECIES OF RHIZOPUS

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INTRODUCTION

A decay of sweet potatoes commonly referred to as soft rot has been attributed by a number of investigators to *Rhizopus nigricans*. The first known report of its occurrence on this crop was made by Halsted (5) in 1890, and since then this organism has been cited as the cause of the decay of a number of other vegetables as well as fruits. Most of the important literature dealing with the decay caused by *R. nigricans* has been previously reviewed by Harter, Weimer and Adams (8) and Stevens and Wilcox (11); hence only some of the crops attacked will be mentioned. They are as follows: tomatoes (*Lycopersicon esculentum*), (2, p., 515-516, 7, 12), potatoes (*Solanum tuberosum*) (9), quince (*Cydonia*) (6), pears (*Pyrus*), raspberries (*Rubus*), currants (*Ribes*), plums (*Prunus*) (2, p. 515-516.) fruits of *Cornus masculus*, *Morus alba* and apple (*Pyrus malus*) (4), figs (*Ficus*) (3), strawberries (*Fragaria*) (10), and red raspberries (11). More recent investigations (1) have shown *Rhizopus nigricans* to be the cause of considerable damage to corn on the seed germinator.

The above references to the literature are su icient to show that *Rhizopus nigricans* is regarded by many investigators as a common cause of the decay of a number of fruits and vegetables. That other species of the genus Rhizopus have not been found to cause decay may be due perhaps in part at least to the facts, first, that *R. nigricans* is probably by far the most common of the parasitic species and, second, to the too common error of classifying all such molds as *Rhizopus nigricans*, without attempting to identify them by comparing with the other species of the genus. Judging from the reports of its isolation from vegetables decayed both in storage houses and on the markets, it is probable that *Rhizopus nigricans* is responsible for more decay than any of the other species. On the other hand, it will be shown that several other members of the genus will rot sweet potatoes. In fact, some of the species which are even more vigorous parasites under artificial conditions than *Rhizopus nigricans* frequently are found, when the isolations are made from fruits and vegetables which are decayed at temperatures above or below the optimum for *Rhizopus nigricans*.

The results of the experiments which are recorded in this paper were derived from a series of inoculations made upon the Yellow Jersey variety of sweet potato with 11 different species of Rhizopus.

METHODS OF TESTING PARASITISM

Harter, Weimer and Adams (8) have shown that infection seldom results when the spores and hyphae of Rhizopus are smeared on the wounded surface of sweet potatoes, confined in a moist chamber. Furthermore, negative results were usually obtained even when the spores were suspended in water in a "well" made in the potato. If on the other hand a 24 to 48 hour growth of the fungus on sweet potato decoction was poured into a "well" made in the potato and the "well" sealed over with a cover slip set in vaseline or plugged with cotton a large percentage of the potatoes decayed. Since this method is the only one which could be relied upon to give anything like uniform results in moist chambers it has been employed in all these experiments. After inoculation the potatoes were incubated at a temperature suitable for the growth of the particular species of Rhizopus with which the inoculations were made. In this connection it should be pointed out that no attempt was made to incubate the potatoes at the exact optimum temperature for growth for the species of Rhizopus under investigation. Similarly to Hanzawa (7) we found that the species studied could be roughly divided into three groups with respect to temperature; i. e., high, medium, and low temperature forms. There is a considerable range of temperature at which the different species of a single group will thrive well. For example, the high temperature forms grow fairly well at temperatures ranging from 30 to 40° C., and some species have a maximum even higher than 40° C. The medium temperature forms grow best at temperatures varying from 20 to 35° C. and the low temperature forms from 15°to 20° C. It will be seen that there is some overlapping, the lowest range given for the high temperature forms being somewhat less than the highest range for the intermediate forms. It should not be inferred, however, that the different species will not grow at a temperature above or below the range given for the group in which they have been placed. Any of the species will survive and make some growth over a much wider range of temperatures than that given for any of the groups, but the growth at temperatures outside the limits given is likely to be abnormal or retarded. In order, therefore, to insure as nearly optimum conditions for infection and decay as possible, the potatoes were incubated at some temperature within the range at which a normal growth of the species under investigation took place.

The inoculation experiments were carried out in duplicate, thirty potatoes being inoculated in each experiment. The checks (30) were treated in every way the same as the inoculated potatoes except that sterile sweet potato decoction was poured into the well.

In from 24 to 48 hours some of the potatoes were partially decayed. If the decay originated at the point of inoculation (well) an isolation was made and the organism identified.

SOURCE OF ORGANISMS

Inoculation experiments were carried out with the following 11 species of Rhizopus: *Rhizopus nigricans* Ehrnb., *R. reflexus* Bainier, *R. chinensis* Saito, *R. tritici* Saito, *R. artocarpi* Racib., *R. delemar* (Boid.) Wehmer and Hanzawa, *R. Maydis* Bruderl., *R. nodosus* Namysl., *R. oryzae* Went and Pr. Geerlings, *R. microsporus* van Tieghem, and *R. arrhizus* Fischer.

The writers obtained all of the species except *nigricans* from Mr. E. D. Eddy, to whom they are indebted also for some preliminary work on the parasitism of some of the species. Mr. Eddy, who was formerly engaged in a monographic study of the genus, collected the various species from different sources. *Rhizopus artocarpi* was obtained from Reinking, Philippine Islands; *R. Maydis, R. nodosus,* and *R. delemar* from Centralstelle für Pilzkulturen, Amsterdam, Holland; *R. tritici, R. oryzae* and *R. chinensis* from Dr. A. F. Blakeslee. *Rhizopus reflexus* and *R. microsporus* were original isolations made by Mr. Eddy from apricots and soil respectively.

Preliminary to the inoculation experiments a pure line culture of each organism was obtained. This was done by growing the fungus on Beyerinck's agar where a comparatively few long strands of hyphae are produced. These are generally so well separated that a cutting can be made readily from a single thread.

EXPERIMENTAL DATA

Before the different species were "pure lined" a series of inoculations were made upon sweet potatoes. It was later concluded to repeat the entire set of experiments using pure line cultures in each case. The results of the earlier experiments which were preliminary in nature are not included in the table for the reason that they would add greatly to the volume of data to be presented without in any way altering the conclusions to be drawn.

DISCUSSION OF RESULTS

The experiments, the results of which are shown in table 1, had for their primary object to determine which of the species of Rhizopus are parasitic on sweet potatoes, and incidentally the degree of parasitism. While some conclusion might be drawn with respect to the relative degree of parasitism of the different species, a strict interpretation of the results as shown by the table would be misleading. At the outset it might be well to call attention to the fact that an attempt was made to study in a very general way the influence of temperature on infection and decay. As previously pointed out the species can be roughly grouped into high, low, and intermediate temperature forms. In some instances

TABLE 1

Showing the number of sweet potatoes infected in each experiment by the different species of Rhizopus and the organism isolated. There were 30 checks and 30 inoculated potatoes in each experiment

I = inoculated, C = check

SPECIES	TEMPERATURE DEGREES C.	NUMBER OF POTATOES DECAYED	ORGANISMS ISOLATED
nigricans	22–26	I. 14 C. 1	R. nigricans 14 do 1
	20	I. 25 C. 1	R. nigricans 25 do 1
reflexus	20	I. 19 C. 1	R. reflexus 19 R. nigricans 1
	20	I. 13 C. 0	R. reflexus 13
chinensis	33–35	I. 7 C. 8	R. tritici group, 7 R. tritici group, 8
	33–35	I. 8 C. 8	R. tritici group, 8 do 8
tritici	22–26	I. 28 C. 0	R. tritici 28
	22–26	I. 30 C. 0	R. tritici 30
oryzae	34	I. 26 C. 0	R. oryzae 25 tritici 1
	30	I. 29 C. 0	R. oryzae 29
microsporus	23	I. 0 C. 0	
	14 & 18	I. 0 C. 0	
artocarpi	22–26	I. 13 C. 0	R. artocarpi 13
	20	I. 21 C. 0	R. artocarpi 13
delemar	22–26	I. 28 C. 0	R. delamar 28
	22–26	I. 20 C. 1	R. delemar 20 R. tritici 1
maydis	22–26	I. 14 C. 0	R. maydis 14
	30	I. 30 C. 0	R. maydis 30
nodosus	34	I. 30 C. 0	R. nodosus 30
	30	I. 30 C. 0	R. nodosus 29 R. delemar 1
arrhizus	30	I. 30 C. 0	R. arrhizus 30

one of the duplicate tests was carried out at a temperature higher than the optimum for the best development of the species under investigation and as a result the percentage of infection was small. There are at least three outstanding examples as shown by the table where the percentage of infection was low because a temperature unfavorable to the growth of the fungus was employed. In the experiments with *Rhizopus nigricans* 14 out of 30 and 25 out of 30 potatoes were infected at temperatures of 22 to 26° C. respectively. Similar results were obtained with *Rhizopus artocarpi*. At a temperature of from 22 to 26° C. only 13 out of 30 potatoes were infected while at 20° C. there were 21. Both *R. nigricans* and *R. artocarpi* are low temperature forms, the percentage of infections decreasing with the increase of temperature above 20° C. It is not unlikely that even a higher percentage of infection could be obtained with both *R. nigricans* and *R. artocarpi* if the potatoes were incubated at a temperature lower than 20° C. *Rhizopus maydis*, however, is a species of quite different temperature requirements. At a temperature of from 22 to 26° C. 14 out of 30 potatoes became infected while at 30° all the potatoes decayed. *Rhizopus nigricans* and *R. artocarpi* being low temperature forms are more virulent at 20° C. or lower. *Rhizopus maydis* being one of the intermediate forms is more parasitic at 30° C. than at 22 to 26° C.

The results show that 9 of the 11 species investigated are parasitic to the sweet potato, the two exceptions being *R. chinensis* and *R. microsporus*. It is not possible to state at this time whether or not there is any variation in the degree of parasitism of the different species and if so to what extent. It might appear from the table that some species were less parasitic than others if judged by the percentage of infection alone. However, in some cases, the low percentage of infection was due to the unfavorable temperature at which the potatoes were incubated and not to any lack of parasitism on the part of the fungus.

It will be noted that the two non-parasitic species *R. chinensis* and *R. microsporus* are high and low temperature forms respectively. In the case of the former some of both the inoculated and check potatoes were decayed but none by *R. microsporus*. Potatoes inoculated with *R. microsporus* were subjected to three different temperatures viz., 14, 18 and 23° C. and in no case did infection result. Higher temperatures were not employed because preliminary work showed that the species thrived in cultures only at these or lower temperatures.

These results show that, not only *Rhizopus nigricans* but other species of the genus Rhizopus may be responsible for the losses of sweet potatoes in storage and in transit. It is not unlikely as the writers hope to show later, that other crops, both fruits and vegetables, may be decayed by other species of Rhizopus than *R. nigricans*. Although *R. nigricans* seems to be the most common species found on sweet potatoes, this ma^

be due to the fact that the potatoes are for the most part held at a temperature more favorable for its growth than for the growth of the other species. When a temperature of 30° C. is reached other species of Rhizopus are found.

SUMMARY

1. The results of the inoculation experiments show the following species of Rhizopus to be parasitic on sweet potatoes: *R. nigricans, R. tritici, R. nodosus, R. maydis, R. reflexus, R. artocarpi, R. delemar, R. arrhizus* and *R. oryzae. Rhizopus chinensis* and *R. microsporus*, the two other species tried, were not parasitic on the sweet potato.

2. The different species can be roughly grouped into high, intermediate and low temperature forms. The high temperature forms thrive best at temperatures varying from 30° to 40° C.; the intermediates, at temperatures varying from 20 to 35° C; and the low temperature forms, at temperatures ranging from 15 to 20° C. It was found that the best results from inoculations were obtained when the potatoes were incubated at the temperature best suited for the growth of the species with which the inoculations were made.

LITERATURE CITED

(1) ADAMS, J. F., AND RUSSELL, A. M. Rhizopus infection of corn on the germinator. Phytopath. **10**: 535–543, *6 fig.* 1920. Literature cited, p. 542–543.

(2) BEHRENS, J. Beiträge zur Kenntnis der Obstfäulnis. Centralbl. Bakt. Parasitenk. u. Infektionskrankh. Abt. 2. **4**: 514–522, 547–553, 577–585, 635–644, 700–706, 739–746, 770–777. 1898. Bibliographical footnotes.

(3) EDGERTON, C. W. Diseases of the fig tree and fruit. Louisiana Agric. Exp. Sta. Bull. 126, *20 p., 8 pl.* 1911. Literature, p. 19

(4) EHRENBERG, C. G. De mycetogenesi Nova Acta Phys. Med. Acad. Caes. Leop. Carol. Nat Cur. **10**: 159–222, *pl. 10–15.* [1821]? (1820.)

(5) HALSTEAD, B. D. Some fungous diseases of the sweet potato. New Jersey Agric. Expt. Sta. Bull. 76, *32 p., 19 fig.* 1890.

(6) HALSTEAD, B. D. Some fungous diseases of the quince fruit. New Jersey Agric. Exp. Sta. Bull. 91, *16 p., 12 fig.* 1892.

(7) HANZAWA, . Studien über einig *Rhizopus*-arten. Mycol. Centbl **5**: 230–246, 257–281, *12 fig* 1914–15

(8) HARTER, L. L., WEIMER, J. L., AND ADAMS, J. M. R. Sweet-potato storage-rots. Jour. Agric. Res. **15**: 337–368, *pl. 21–27.* 1918. Literature cited, p. 366–368.

(9) ORTON, W. A. Potato diseases in San Joaquin County, California. United States Dept. Agric. Bur. Plant Indus. Circ. 23, *14 p.* 1909.

(10) STEVENS, F. L., AND PETERSON, ALVAH. Some new strawberry fungi. Phytopath. **6**: 258–267, *26 fig.* 1916.

(11) STEVENS, N. E., AND WILCOX, R. B. Rhizopus rot of strawberries in transit. United States Dept. Agric. Bull. 531, *22 p.* 1917. Literature cited, p. 21–22

(12) WORMALD, H. Experiments with *Rhizopus nigricans* on tomatoes. Jour. South-Eastern Agric. Coll Wye. **1**: 381–391, *fig. 5–6.* 1912.

BRIEFER ARTICLES

LOSS FROM RYE ERGOT[1]

EDITH K. SEYMOUR AND FRANK T. MCFARLAND

WITH TWO FIGURES IN THE TEXT

It is ordinarly assumed that the loss caused by ergot (*Claviceps purpurea* (Fr.) Tul.) in rye is directly proportional to the number of sclerotia produced. Indeed, it might be thought at first that it is represented merely by the transformation of this number of potential rye kernels into the corresponding number of worthless or poisonous sclerotia. As a matter of fact, however, field observations soon convince one that the actual damage must exceed this. The developmental processes of the parasitized inflorescences are evidently fundamentally altered. This may be attributed in part to the unusually heavy drain which the developing sclerotia may make upon the nutrition supply of the host and in part to the damage caused by the parasitic hyphae penetrating various parts of the spikes. As a result of this parasitism, there may be produced, besides the sclerotia, numerous blasted kernels and empty florets.

In order to secure evidence bearing upon these matters, observations which were initiated by Dr. L. R. Jones in the summer of 1918 have been supplemented and continued by the writers during 1919 and 1920. The results are tabulated in table 1. All observations were made on winter rye except when otherwise designated in the table. Counts in 1918 show that when there were only 3 per cent of the florets containing sclerotia there were also 35 per cent either empty or filled with blasted kernels. In 1919, three more series of observations were made. In one series in which 14 per cent of the florets contained sclerotia, 64 per cent in addition either held blasted kernels or were empty. In the second series where there were 21 per cent with sclerotia, there were 66 per cent either empty or holding blasted kernels. It should be noted here that it has been widely observed that there is a high degree of sterility in rye and so the question has arisen whether this blasting and lack of kernel development is due to the presence of the fungus or to some other factor. When the last 1919 observations on ergotized rye were made, control observations were also made on unergotized spikes, collected not only from the same field but also in close proximity to the ergotized spikes. The third

[1] Co-operative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

series of counts with 3 per cent of the florets containing sclerotia showed 38 per cent either empty or holding blasted kernels, whereas, in the control, the number of blasted kernels or empty florets was only 33 per cent.

In the three observations made in 1920, it was evident from the occurrence of the sphacelial exudate that the fungus may attack the spikes

TABLE 1

Loss from rye ergot due to destruction or blasting of kernels
(All collections were of winter rye except the last two)

PLACE AND YEAR OF COLLECTIONS	ERGOTIZED OR UNERGOTIZED SPIKES	NUMBER OF SPIKES COUNTED	NUMBER OF NORMAL KERNELS	PER CENT. OF NORMAL KERNELS	NUMBER OF BLASTED KERNELS OR EMPTY FLORETS	PER CENT OF BLASTED KERNELS OR EMPTY FLORETS	NUMBER OF SCLEROTIA	PER CENT. OF SCLEROTIA	AVE. LENGTH (CM.) PER SPIKE	AVE. WEIGHT (GM.) PER SPIKE
Ellison Bay, 1918	ergotized	40	965	62	543	35	44	3		
Madison, 1919	"	52	531	22	1660	64	375	14		
Madison, 1919	"	21	175	13	902	66	282	21		
Edwardsville, 1919	"	74	2661	59	1709	38	154	3	10.3	0.9
Edwardsville, 1919	unergotized	74	3291	67	1613	33	0	0	11.6	1.4
Madison, 1920	ergotized	182	2252	24	5484	57	1797	19	10.3	0.9
Madison, 1920	unergotized	239	10152	68	4590	31	0	0	10.8	1.4
Burke, 1920	ergotized	164	5497	55	3744	38	661	7	10.8	0.8
Burke, 1920	unergotized	139	5845	71	2375	29	0	0	10.7	0.6
Madison, 1920 (spring)	ergotized	197	4634	47	4580	47	575	6	8.9	0.8
Madison, 1920 (spring)	unergotized	199	7223	68	3479	32	0	0	9.7	1.2

without producing sclerotia. To obviate the danger of counting parasitized spikes as normal, 1120 spikes were tagged and numbered in the field at the time when the development of the sphacelial exudate was at its height. Of this number, 543 were ergotized, as evidenced by the presence of the exudate, and 577 were unergotized, that is, they showed no exudate. The unergotized spikes were examined about every other day to make sure that no later infection took place. When such infection did take place as evidenced by the appearance of the exudate, the newly infected spikes were included with the diseased number. The attempt was made to tag one ergotized spike and a corresponding unergotized spike in close proximity so that the two spikes might be subjected to similar environmental conditons. These spikes were found in three different localities. In the first in which 19 per cent of the florets held selerotia, 57 per cent were empty or contained blasted kernels, whereas,

the percentage of blasted kernels or empty florets was lower in the control by 26 per cent. In the second locality where 7 per cent of the florets held sclerotia, 38 per cent were empty or held blasted kernels, with a control in which 29 per cent were similarly defective. In the third locality, sclerotia were found in 6 per cent of the florets, whereas 47 per cent of them were empty or contained blasted kerels, with a control showing 15 per cent less of similarly defective florets.

These observations of fungal destruction were further verified by examination of the florets for the presence of conidia, this being regarded as indicative of mycelial penetration. Of 24 spikes collected in Edwardsville, 30 per cent of the florets showed no conidia or sclerotia, 49 per cent

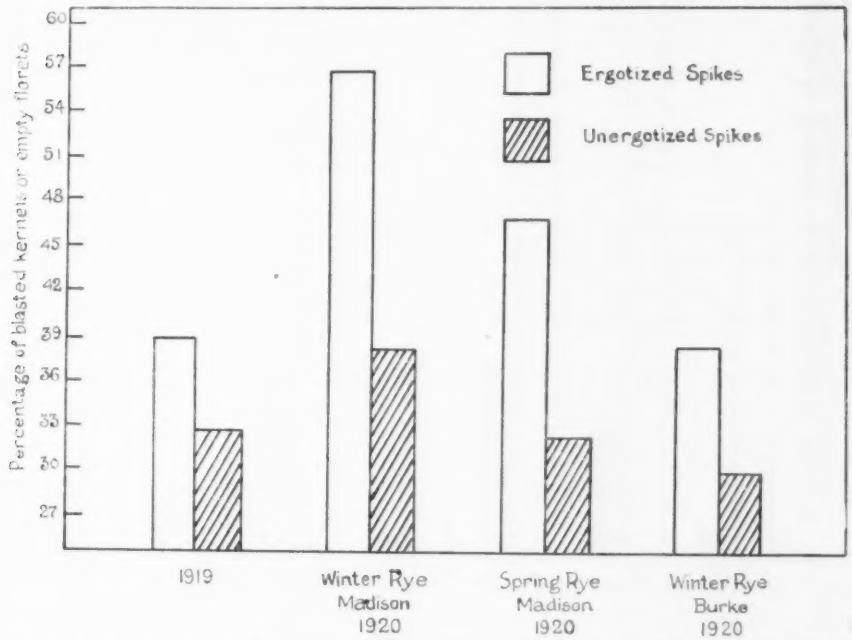

Fig. 1. Graph showing percentages of blasted kernels and empty florets, and sclerotia bearing florets in seven fields of rye.

showed abundant conidia, and 21 per cent showed sclerotia. Thus 70 per cent of the florets were infected by the fungus, although only 21 per cent contained sclerotia. Examination of 894 blasted kernels or empty florets in 20 spikes of this collection showed 64 per cent with abundant conidia, whereas only 36 per cent contained no conidia.

In order to obtain all possible data on the effect of the fungus, other observations were made. The average length and weight, sclerotia included, of the ergotized spikes was compared with that of the unergotized Of the material collected in Edwardsville, the average unergotized spike was 1.3 cm. longer and weighed 0.5 gm. more than the average ergotized spike; of the winter rye from Madison in 1920 the ergot-free spike was 0.5 cm. longer and weighed 0.5 gm. more than the infected spike; and of

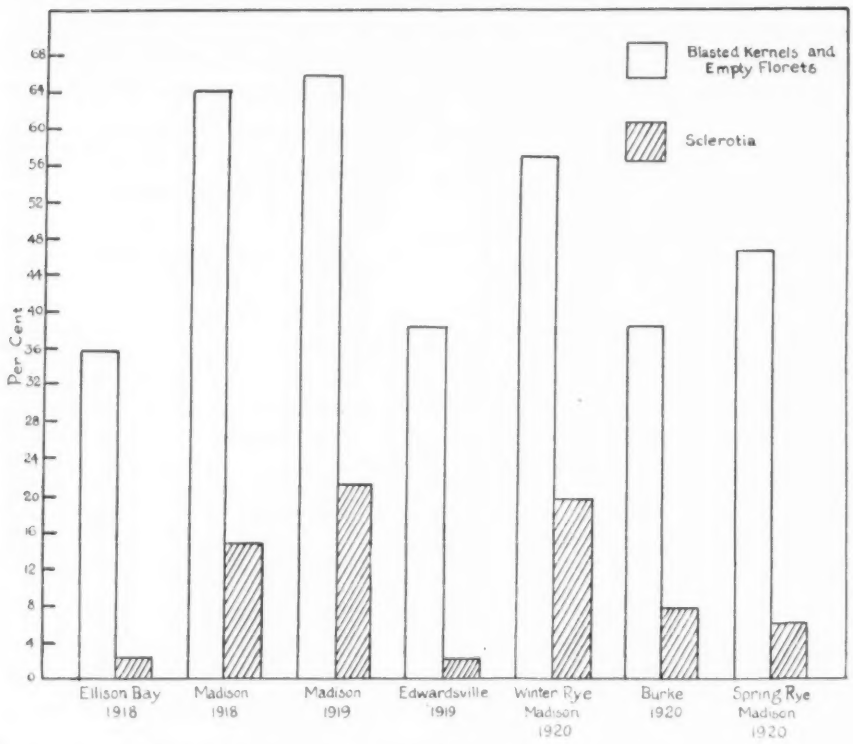

Fig. 2. Graph showing the percentages of blasted kernels and empty florets in ergotized and in unergotized heads of rye.

the spring rye collected at Madison in 1920 the unergotized spike was 0.8 cm. longer and weighed 0.4 gm. more than the ergotized spike. On the other hand, in the material from Burke, the average ergotized spike was 0.1 cm. longer and weighed 0.2 gm. more than the average unergotized spike. Our results show a shorter ergotized axis except in the case of the Burke material. If infection can take place in the oldest ovaries before the spike has attained its full growth, it is conceivable that such a retarda-

tion might be due to the fungus. In regard to the weight, even including the sclerotia, our results show a slight decrease in every case except the last.

The question of the maturity of infected and uninfected rye plants has arisen. In looking over a rye field one notices that the ergotized plants, as a rule, are later in their development than the unergotized. This effect may possibly be due to the attack of the plants normally later in maturing or to the fungus retarding the filling of the kernels. To throw some light upon whether the fungus attacks less mature spikes or retards the ripening of ergotized spikes, counts were made of the material collected at Edwardsville, which shows the following conditions:

Total number of ergotized spikes	76	100%
Number of ergotized spikes immature	53	69%
Numbes of ergotized spikes mature	23	31%
Total number of unergotized spikes	120	100%
Number of unergotized spikes immature	43	36%
Number of unergotized spikes mature	77	64%

As great care was taken to collect infected plants grown under the same conditions as the unergotized plants, our counts are significant and show that the greater percentage of infected spikes are immature. This, however, does not show whether the fungus is responsible for this delay in maturity.

In summarizing, the average of the counts may be expressed as follows:

SPIKES	PER CENT. NORMAL KERNELS	PER CENT. BLASTED KERNELS OR EMPTY FLORETS
Ergotized	43	48
Unergotized	69	31

This tabulated average and figure 1 show clearly that in ergotized spikes there is, beside the percentage of florets containing sclerotia, a much larger percentage of florets containing neither sclerotia nor normal kernels. Further, there is always a greater percentage of these latter florets in the ergotized spikes than in the unergotized spikes (Fig. 2). Lastly, it is evident in the diseased plants that the majority of the blasted kernels or empty florets have been penetrated by the fungus. Thus it may be concluded that, due to the presence of the ergot fungus, there is a marked reduction in the yield of normal kernels beyond the simple replacement of kernels by sclerotia.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE
AND WISCONSIN AGRICULTURAL EXPERIMENT STATION.

THE BLOSSOM BLIGHT OF THE PEACH

MEL. T. COOK[1]

WITH PLATE XII

The *blossom blight* form of the brown rot (*Sclerotinia cinerea* (Bon.) Schröt.), has been known for a number of years but since it is not of annual occurrence in a severe form, its importance has been overshadowed by the *fruit rot* form which occurs later in the season. The *blossom blight* form of the disease is quite common in New Jersey and during the blossoming seasons of 1919 and 1920 was exceptionally severe. These two outbreaks lead the author to begin some studies which were reported at the 1920 December meeting of the American Phytopathological Society. Requests from members of the Society have induced the writer to make this preliminary report at this time.

The severity of this outbreak raised two questions: (1) The source of the infection, and (2) the most satisfactory methods of control. The life history of the organism very naturally led to the examination of the mummied fruits for Monilia and apothecia. The presence of either might be sufficient to explain the infection, but the latter is considered by many to be of major importance. The section of the state in which the disease was most severe includes a large number of orchards of various ages and sizes, and in various conditions so far as cultivation and fertilization are concerned. Therefore, the conditions for study were exceptionally favorable. A very careful study of this territory was made in both 1919 and 1920. The organism was found to be viable on many old mummies but not a single specimen of the apothecial stage could be found. However, it was impossible to correlate the disease with the presence or absence of the mummies or with the cultural conditions of the orchard. Among these orchards was one that had been planted and cared for by the Horticultural Department of the New Jersey Agricultural Experiment Station and in which it was almost impossible to find mummies of any kind. Yet a large majority of the blossoms were destroyed in 1919 and the infestation was very severe in 1920. Many other orchards in which the mummies were rare were as heavily infected as orchards in which they were abundant. In fact the studies during the past two years have indicated that the infections were due to some source other than either mummies or the apothecia.

Further studies indicated that small cankers formed at the base of the

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In most cases only a few buds at the base of the shoot are infected, while in other cases as many as 75 per cent of them become infected. In some cases the base of the shoot is girdled and the entire shoot dies giving an appearance very similar to that of fire blight on the pear.

The cankers usually appear just below and at the base of the bud; they enlarge very slowly during the growing season, sometimes completely involving and killing the buds, but in most cases the buds live and produce flowers the following season. The living organism can be isolated from many of these cankers during the summer and winter. The fungus develops from these cankers during the next blooming season and if the weather is warm and moist, appears as grayish, tufted masses, bearing a great abundance of conidia. This has been the most important source of blossom infection during 1919 and 1920.

In many cases the fungus works its way outward through the pedicel and into the flowers, while in other cases the blossoms become infected from spores and the fungus works its way back into the wood and gives rise to a canker. This latter form of infection has been noted by many workers but the formation of the canker on the new growth a year in advance of blooming and thus becoming a source of infection appears to have been very generally overlooked. A brief review of the literature on this point is as follows:

In 1892 Chester (1, p. 6) wrote "This blight of the blossom had extended so far as to cause in many cases, a browning of the wood at the base of each blighted blossom."

In 1899 Cordley (2, p. 8-9) wrote "Upon the peaches and cherries the fungus usually makes its first appearance in the spring upon the flowers, about the time the petals fall." * * * * *

"In the peach, the blossoms of which have very short pedicels, the blight does not stop with the destruction of the flower, but the mycelium of the fungus may extend through the pedicel into the tissues of the twig. The portion of the twig thus attacked soon assumes the characteristic leathery brown color of brown rot. The extent of the tissue thus bud during the preceeding year were the most important factor in the the development of this stage of the disease. These cankers originate as soon as the leaf shots begin to develop, which is about the time or immediately following the opening of the flower buds. The young wood of these leafy shoots is very tender and appears to be very easily infected at this time. If the weather conditions are favorable for the development of the blossom blight, they are also favorable for the formation of new cankers. The first infections are at the bases of the shoots, other buds becoming infected in more or less regular succession throughout the season, the number depending on favorable or unfavorable conditions.

involved usually varies with the conditions of heat and moisture, but should it extend around the twig so that the latter is girdled, all of the terminal portion beyond the point of infection will blight."

Quaintance (6, p. 247, 248) in 1900 says "In the peach, particularly, the fungus does not necessarily stop its growth in the flowers, but ths mycelium may grow down through the flower stalks and attack the tissues of the twigs."

Scott & Ayres (7, p. 11) reported in 1910 that, "The fungus also attacks the blossoms and twigs, thus often destroying a portion of the fruit crop at blooming time. The diseased blossoms turn brown and become dried, adhering to the twigs for some weeks. The fungus may extend from the dead blossoms into the bark, forming a small brown canker which frequently girdles the twig. In low, damp situations, specially in a wet spring many blossoms and fruit bearing twigs may thus be destroyed."

Jehle in 1913 (4) says—"Sometimes the shuck becomes diseased and this is then shed without further injury to the pistil, but usually the entire flower becomes infected and the disease travels down the pedicel of the peach flower, finally entering the fruit spur, causing there the exudation of a large quantity of gum, which surrounds the blossom and holds it fast to the spur. Such diseased blossoms remain clinging to the fruit spurs during the entire summer, and whenever there is a sufficient moisture the typical ash grey conidia are produced in great abundance on the fruit."

In 1915 Heald (3) wrote "In case of blossom infection the fungus may advance into the fruit spurs and cause some blighting, although in the majority of flower infections the fungus does not penetrate farther than the base of the flower pedicel."

It appears that the appearance of cankers on new wood and the relation of these cankers to the infections resulting in the "blossom blight" has been very generally overlooked. It also appears that the workers have very generally attributed the formation of cankers to infected blossoms.

The flower having been killed regardless of time or source of infection the bark splits and a large gummy canker is produced which may continue active for a number of years or which may heal and form a more or less prominent scar. The writer is uncertain as to the length of time that these cankers may presist as important centers for the development of spores. When first formed they are capable of producing great numbers of spores if the weather conditions are favorable. As the season advanced they produced fewer and fewer spores, many of them ceasing to produce spores and healing completely. However, it is evident that the fungus in some of them may live for one or more years and continue to be foci or infection. These cankers have been mentioned by several workers.

BLOSSOM BLIGHT OF THE PEACH DUE TO SCLEROTINIA CINEREA. NOTE THE CANKERS AT BASES OF BUDS

Jehle (4) in 1913 and 1914 stated that "The fungus remained alive in the cankers during the winter and only needed favorable conditions to call it forth into activity." He also says "A callus is soon formed outside of the diseased area and sometimes the wound heals over, but usually this callus becomes diseased. The following year healing is again attempted and the callus usually is again invaded. This process is repeated year after year so that several such calli are formed. The writer has seen as many as five on a single canker."

Heald (3) in 1915 stated that "the fungus works down through the blossom or fruit spur into the bark and spreads to form the canker. Such cankers serve, in the same way as hold over fire-blight cankers, as centers from which new infections may originate." He also says "such cankers may continue to extend for a perod of years before the branch is girdled or the progress of the lesion checked."

In 1918 McCubbin (5) in his paper on "Peach Canker" in which he considered *Valsa leucostoma* as the factor of greatest importance says, "As far as we can see, the Brown Rot fungus acts as an initial agent only, and while it may readily bring about the first stage of cankers it is considered to play little if any part in the second stage—the succeeding yearly extension of these."

The writer is uncertain as to the length of time that the fungus will live in the cankers but it is very evident that they are of greatest importance immediately before and immediately following the blossoming and that they become of less and less importance as the season advances. The fungus undoubtedly dies in most of them during this first season. The writer is also uncertain as to the importance of *Valsa leucostoma* and other organisms in perpetuating these cankers.

SUMMARY

1. Cankers formed on the new growth are the most important sources of infection resulting in the "blossom blight" of the peach.

2. The fungus emerging from the spores in grayish, tufted masses produces an abundance of conidia spores.

3. The blossom infection may be from these spores or by a growth of the fungus from the cankers through the pedicel and into the flower.

4. Cankers may also be produced by growth from the blossom through the pedicel and into the wood at the base.

5. Immediately following the blossoming, the cankers, split, produce an abundance of gum and if the conditions are favorable an abundance of spores. They become of less and less importance in the distribution of spores as the season advances.

6. Many of these cankers heal but some of them presist for one or more years and may continue to produce spores but are of minor importance when compared with their activity during the blossoming season.

7. The cankers are of much greater importance in New Jersey than either the Monilia as found on the mummies or the apothecia.

LITERATURE CITED

(1) CHESTER, FREDERICK D. Can peach rot be controlled by spraying? A preliminary report. Delaware Agric. Exp. Sta. Bull. 19, *16 p., illus.* 1892.
(2) CORDLEY, A. B. Brown rot. Oregon Agric. Exp. Sta. Bull. 57, *15 p., 7 fig., 1 pl.* 1899.
(3) HEALD, F. D. Brown rot of stone fruits. Washington Agric. **8**: 9–14. 1915.
(4) JEHLE, R. A. The brown rot canker of the peach. Phytopath. **3**: 105–110, *pl. 10.* 1913. Literature cited, p. 110.
(5) MCCUBBIN, WILLIAM A. Peach canker. Canada Agric. Exp. Farms Bull., 2d ser., 37, *0 p., 6 pl.* 1918.
(6) QUAINTANCE, A. L. The brown rot of peaches, plums and other fruits. Monilia fructigena Persoon. Georgia Agric. Exp. Sta. Bull. **50**: 237–269, *9 fig.* 1900.
(7) SCOTT, W. M., AND AYRES, T. WILLARD. The control of peach brown-rot and scab. United States Dept. Agric. Bur. Plant Ind. Bull., 174, *31 p., 1 fig., 4 pl* 1910.

CENANGIUM PINIPHILUM N. SP., AN UNDESCRIBED CANKER-FORMING FUNGUS ON PINUS PONDEROSA AND P. CONTORTA

JAMES R. WEIR

WITH PLATE XIII AND TWO FIGURES IN THE TEXT

The species described below was first collected at Boulder, Montana, on *Pinus contorta,* on June 8, 1915. Since then it has been found abun-

FIG. 1. APOTHECIA OF CENANGIUM PINIPHILUM ON PINUS CONTORTA

dantly in the lake region of northern Idaho, where it attacks *Pinus ponderosa* and *P. contorta*. Since the fungus, in its minute and gross characters, is closer to Cenangium than any genus so far described as parasitic on conifers, it is here described as *Cenangium piniphilum* n. sp., having the following characteristics:

Apothecia appearing singly, then in groups, rupturing the epidermis, at first rounded or ellipsoidal, closed, then cone-shaped, tapering to a stem-like base, expanding and becoming almost plane with the margin, irregularly incurved, but not wholly closed when dry, coriaceous, membranaceous, 2–5 mm. across, carbonous; disc brownish to black, surface velvety or wrinkled; asci clavate, rounded above, long stipitate, averaging 135.8 x 14.1μ, 8-spored; spores irregularly 2-seriate, sometimes 1-seriate, oblong to ellipsoid or fusiform, extremities acute, usually with a single central oil drop, occasionally with two, hyaline, continuous, averaging 18.9 x 6.4 μ; paraphyses filamentous, branched, longer than the asci, hyaline.

Type collected on a 15-year tree of *Pinus contorta* at Priest River, Idaho, in the Kaniksu National Forest, May 12, 1920, common throughout northern Idaho, eastern Washington and western Montana. Type material deposited in the Office of Pathological Collections, and Office of Investigations in Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. (Weir Herb. 14973).

This fungus, which is of considerable silvicultural significance, attacks *Pinus ponderosa* and *P. contorta* between the ages of 5 to 25 years, or older. Infection usually occurs at the nodes, less frequently on the internodes, but may eventually spread throughout the entire length of the stem. The mycelium penetrates the cortex, phloem, and wood, and causes a canker (Plate XIII, fig. A). The cambium is killed, preventing any further growth in thickness at the point of infection, and witheach year's increment the outline of the tree in cross section becomes more irregular (Pl. XIII, fig. B). The penetration of the mycelium into the cortex, phloem, and wood causes the exudation of large quantities of resin on the surface of the canker, which runs down the bark very conspicuously (Pl. XIII, fig. A). The dark brown, extensively branched mycelium following chiefly the medullary rays in the wood (Pl. XIII, fig. C) imparts to it a grayish or bluish-black color (Pl. XIII, fig. B) resembling that caused by *Ceratostomella pilifera* (Fr.) Wint. This color may extend to varying depths or entirely through the tree in the case of two oppositely arranged cankers (Pl. XIII, fig. B). Thecolor, which is always indicative of the presence of mycelium, may

FIG. 2. MATURE ASCI ASCOSPORES, AND PARAPHYSES OF CENANGIUM PINIPHILUM.

extend from one canker to another up and down the tree and is very conspicuous in longitudinal section. Brownish-black deposits in the various cells, especially in the resin passages and medullary rays, intensify the color (Pl. XIII, figs. C, D). The mycelium grows both in the cells and in the intercellular spaces. The stalked apothecia (Fig. 1), containing long stipitate asci with 8 oblong spores (Fig. 2), are borne on the dead bark of the canker and vary in size according to the age of infection. Thefact that infection usually occurs at the branch whorls indicates a natural weakness at this point and may not be due to wounds. Wounds caused by various agents are, however, a source of infection, as has been demonstrated by experiments. The transfer of spores to incisions in the bark of young trees always result in infections. Such infections as occur at the base of branches spread to the main trunk, frequently involving the entire circumference at this point. The number of cankers on a single tree is in some cases limited only by the number of whorls. A 15-year-old tree may have as high as 10, or more, separate and distinct cankers. All of these cankers may be united internally by discolored wood. A thin ridge of tissue winding from one canker to another may be all that remains uninfected.

Although the fungus grows readily on artificial media, it has not yet been induced to form apothecia. The mycelium on artificial media breaks up into conidia, a condition not observed in nature.

The fungus thrives best in dense, moist stands, where the reproduction is over crowded.

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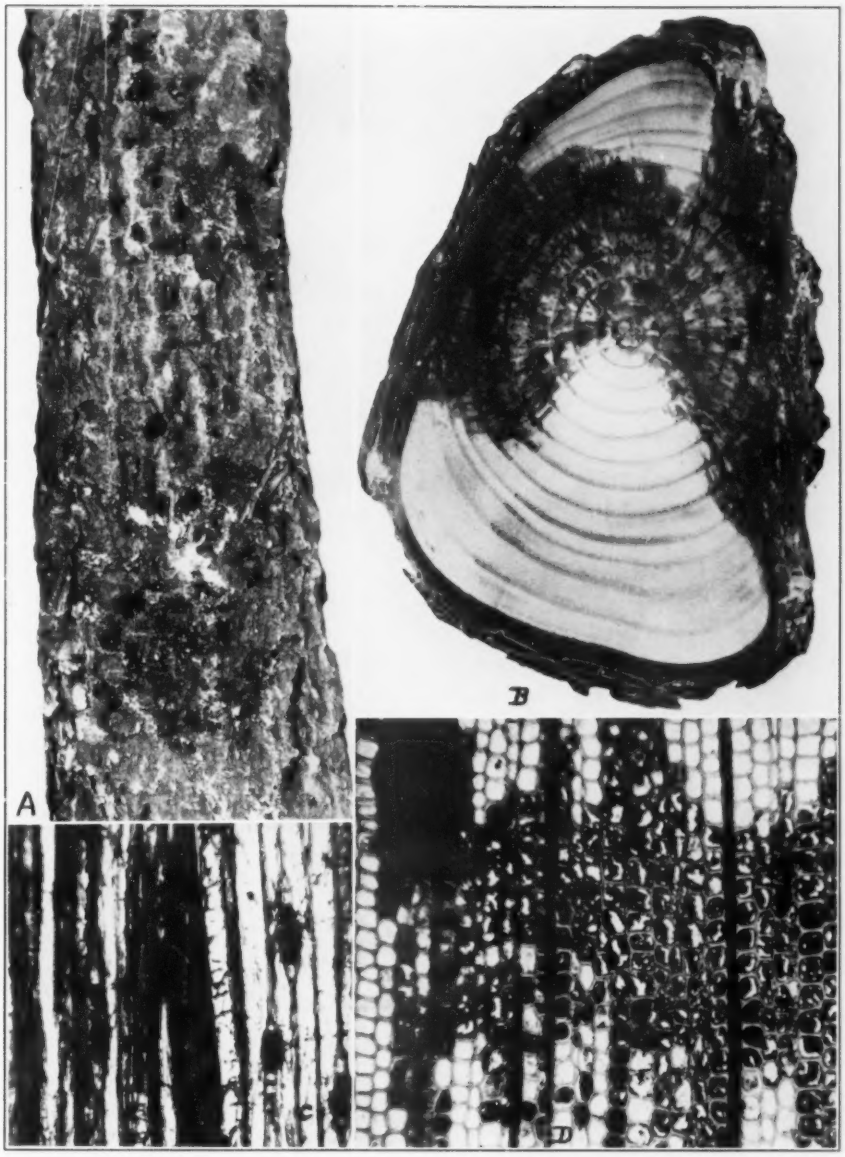

CENANGIUM PINIPHILUM N. SP.

Fig. A Canker on *Pinus contorta* caused by *Cenangium piniphilum*, showing apothecia and exudation of resin; Fig. B. Cross section of canker on *Pinus contorta* showing irregularity and discoloration of infected wood. × 1½; Fig. C. Tangential section of wood of *Pinus contorta* attacked by *C. piniphilum*. Note dark contents of the tracheids and medullary rays. × 110; Fig. D. Cross section of infected wood showing dark contents of the resin canals, medullary rays and tracheids. × 110.

PHYTOPATHOLOGICAL NOTES

Angular leaf-spot of cucumber.—During the summer of 1919, an experimental planting of a strain of pickling cucumber was made at Ellison Bay, Wis., in a soil which, so far as known, had never before grown cucumbers. The primary purpose of this was to determine whether or not the mosaic disease might overwinter with the seed. To this end, the seed had been collected the year before, largely with the assistance of Dr. M. W. Gardner, at Grass Lake, Mich. In each collection the fruits were taken from mosaic plants. No record was made of the occurrence of other diseases on these mother plants but angular leaf spot is known to have been severe in the fields where seed was collected.

Although the results were purely negative as regards mosaic, there was a very interesting development as regards the angular leaf spot. These plants were watched critically, the field being gone over plant by plant at frequent intervals during the season. On July 24, following a few days of warm, misty weather, including one or two gentle showers, there appeared a considerable development of angular leaf spot upon a single hill in the interior of the field. There were two plants in the hill, the disease at this stage appearing simultaneously upon leaves of both. From this as a center, the disease spread rapidly during the next fortnight, the dissemination being essentially radial, probably as a result of the spattering of rainwater, since no insects were in evidence and there was no promiscuous picking or other passing through the field.

Before the end of August, the disease was in evidence over much of this field, comprising nearly one-half acre. One of the most striking things was that although the damage from foliage destruction threatened to be quite serious under the favorable moist weather conditions of the first two weeks, with the subsequent advent of a fortnight or more of dry weather, this type of development was so much inhibited as to become of relatively minor practical importance. On the other hand, before the end of August the development of fruit infection proved very serious indeed. This fruit infection ultimately became so prevalent as to include the appearance of watersoaked spots upon the fruits before they were a quarter grown, and by the time they reached the larger pickling sizes they were often ruined. The two things, then, of special significance from these observations, as supplementing those recorded in the publications of Carsner (Jour. Agric. Res. **15**: 201–220, 1918) and Gilbert and Gardner (Phytopath. **8**: 29–33, 1908), are the fact of its overwintering

on the seed and its serious destructiveness on the young fruit in the field. —L. R. JONES AND S. P. DOOLITTLE.

Field tests with cucumber angular leaf-spot and anthracnose.—During 1918, 1919 and 1920, field tests bearing upon the overwintering of the organisms causing angular leaf-spot and anthracnose of cucumber were conducted on a seed farm at Grass Lake, Michigan, and in 1918 at Plymouth, Indiana.

The effectiveness of seed disinfection in mercury bichloride, 1-1000, for five minutes as a control for the angular leaf-spot[1] was further demonstrated. This treatment does not eliminate absolutely all of the infection, but for practical purposes is very effective since it materially reduces the number of original centers of infection, delays the appearance of the disease and prevents its occurrence in epiphytotic form. At Plymouth, Indiana, anthracnose was rather abundant in a plot planted with untreated seed, while only a trace appeared in a similar plot planted with treated seed. Large scale seed disinfection has been practiced by the H. J. Heinz Company for the last three years with very satisfactory results. The cucumber seed is assembled at a branch factory in quantities up to several tons where it is treated in barrels in fifty-pound lots and spread out over a large floor space to dry.

The Grass Lake field tests indicate that the angular leaf-spot organism (*Bacterium lachrymans* E. F. S. and Bryan) survived twenty months but not thirty-two months on the seed. Thus two-year-old seed cannot be considered free from this organism and should be treated, while three-year-old seed apparently does not carry infection.

Evidence that this organism did not persist in the soil over the winter of 1918-19 was also obtained. Part of a field in which angular leaf-spot was severe in 1918 was planted with disease-free seed in 1919 and none of the disease appeared. Another portion of this field was planted with beans in 1919 and cucumbers again in 1920 and no angular leaf-spot appeared in this crop. Apparently soil infestation is not a great danger in the case of this disease.

As previously reported, the anthracnose fungus (*Colletotrichum lagenarium* (Pass.) Ell. and Hals.) was found to persist one winter in the field.[2] This field in which anthracnose was present in 1917 and 1918 was planted with a non-cucurbitaceous crop in 1919 and then with cucumbers again in 1920. No anthracnose appeared in 1920 which indicates that the

[1] Gilbert, W. W., and Gardner, M. W. Seed treatment control and overwintering of cucumber angular leaf-spot. Phytopathology **8**: 229–233. 1918

[2] Gardner, M. W. Anthracnose of cucurbits. U. S. Dept. Agric. Bull. 727: 58. 1918.

fungus did not persist twenty months in the soil. Thus a two-year rotation should eliminate anthracnose soil infestation.—MAX W. GARDNER AND W. W. GILBERT.

Overwintering of the bacterial wilt of cucurbits.—During the course of studies of cucurbit mosaic conducted at Madison, Wisconsin, by the U. S. Department of Agriculture in cooperation with the Department of Plant Pathology of the University of Wisconsin, trials were made each year from 1917 to 1920 to determine whether the striped beetle, *Diabrotica vittata*, might be an agency in the overwintering of the mosaic disease. While no evidence has been secured which indicates that the insect is concerned in mosaic overwintering, there have been interesting results in regard to its relation to the overwintering of the bacterial wilt, caused by *Bacillus tracheiphilus* Erw. Sm.

In the spring of 1917, approximately 300 beetles were collected in the open field between May 25 and June 6, at the time they first appeared and before any cultivated cucurbits had been planted in the vicinity. One lot of 50 of these beetles was placed in a cage in the greenhouse together with 0 healthy young cucumber plants of small size. Between June 03 and 05 four of the plants showed definite symptoms of bacterial wilt, the cut stems in all cases having the stringy ooze characteristic of the disease.

During the spring of 1919, similar results were again obtained in the course of the trials, about 1000 beetles being tested between April 29 and May 29. On May 25, 50 beetles were collected in the field and placed in the greenhouse in a cage containing 22 healthy cucumber plants each having from four to six leaves. On June 7 three of these plants developed the symptoms of bacterial wilt and were at once removed, but no further wilt infection occurred. No wilt developed during any other of the trials made with the striped beetle during 1919.

No other cases of bacterial wilt have been noted in the greenhouse at Madison during the past four years, and at the time the beetles were collected, both in 1917 and 1919, no cultivated cucurbits had appeared in the neighborhood. Although the striped beetle feeds on the wild cucumber, *Micrampelis lobata*, prior to the appearance of other cucurbits, no signs of wilt have ever been observed either on this host or on *Sicyos angulatus*, which also occurs in the vicinity, and, while the Micrampelis is known to be an agency in overwintering cucurbit mosaic, there has been no evidence to indicate that it is concerned in the case of bacterial wilt.

It has already been shown by Rand and Enlows (U. S. Dept. Agric. Bull. 828, 43 p. 1920) and Rand and Cash (Phytopathology **10**: 132, 1920) that the striped beetle probably carried the wilt organism through the winter, and the above observations seem to support their findings in this regard.—S. P. DOOLITTLE.

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